

Techno-Economic Analysis and Process Intensification of a Cost-Effective Hybrid-Mixing Internally Illuminated Photobioreactor for Improved Microalgal Biodiesel Production

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TECHNO-ECONOMIC ANALYSIS AND PROCESS INTENSIFICATION OF A COST-EFFECTIVE HYBRID-MIXING INTERNALLY ILLUMINATED PHOTOBIOREACTOR FOR IMPROVED MICROALGAL BIODIESEL PRODUCTION

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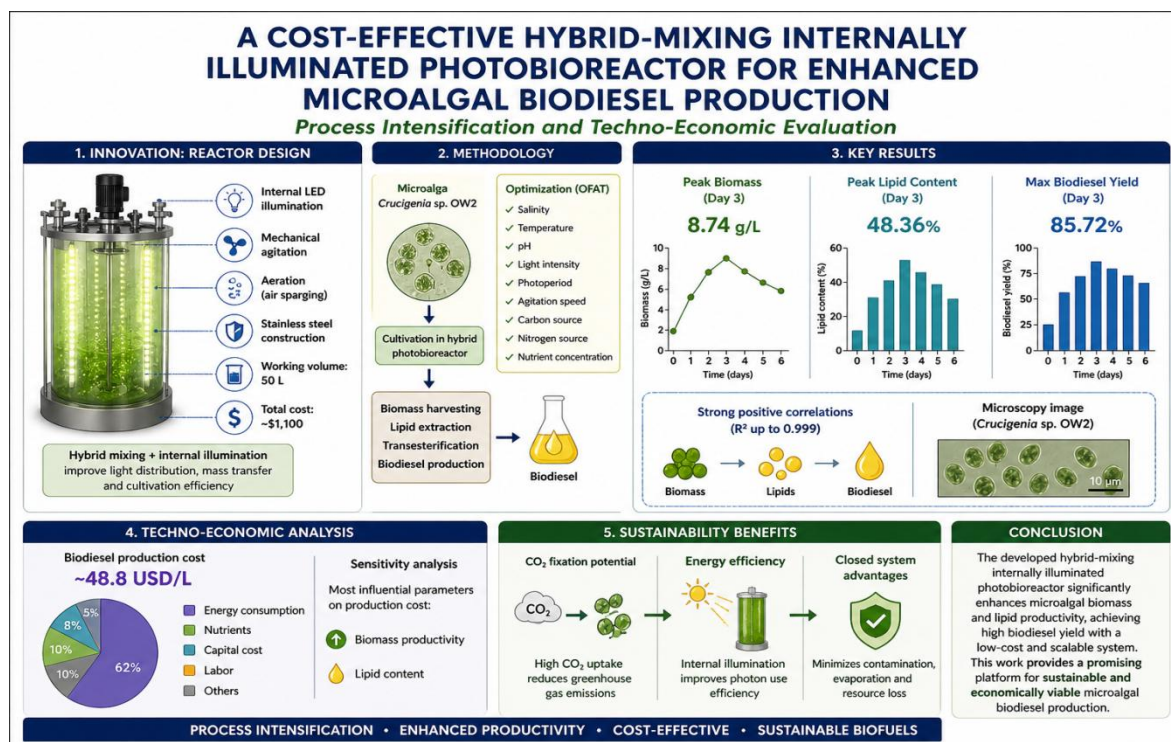
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Highlights:

- Hybrid internally illuminated photobioreactor improves efficiency
- Integrated mixing enhanced biomass and lipid productivity
- Peak biomass 8.74 g/L and lipid content 48.36% achieved
- Biodiesel yield of 85.72% obtained within short cultivation time
- Techno-economic analysis shows cost reduction potential

Graphical Abstract



Abstract

The high cost and limited efficiency of conventional photobioreactors remain major challenges for the large-scale production of microalgal biodiesel. This study aims to develop and evaluate a cost-effective hybrid-mixing internally illuminated photobioreactor to enhance biomass and lipid productivity for sustainable biofuel generation. A 50 L cylindrical stainless-steel photobioreactor integrating internal LED illumination and a hybrid mixing system (mechanical agitation combined with aeration) was designed and fabricated to improve light distribution and mass transfer. The green microalga *Crucigenia* sp. OW2 was cultivated under optimized environmental and nutritional conditions using a one-factor-at-a-time approach, and statistical validation was conducted using one-way ANOVA. Under optimized conditions, maximum biomass concentration

and lipid content reached 6.30 g/L and 36%, respectively. Time-course cultivation in the developed reactor showed peak biomass (8.74 g/L), lipid content (48.36%), and biodiesel yield (85.72%) on day 3. Strong positive correlations (R^2 up to 0.999) between biomass, lipid concentration, and biodiesel yield confirmed efficient conversion pathways. The improved performance is attributed to enhanced photon utilization and gas–liquid mass transfer within the reactor system. A preliminary techno-economic analysis estimated a biodiesel production cost of approximately 48.8 USD/L, primarily influenced by energy consumption and system scale. Sensitivity analysis identified biomass productivity and lipid content as key cost drivers. Overall, the developed system demonstrates a scalable and efficient platform for microalgal biodiesel production, with strong potential for cost reduction through process intensification and scale-up.

Keywords: Microalgal biodiesel, *Crucigenia* sp. OW2, Photobioreactor design, Process intensification, Techno-economic analysis, Lipid accumulation

1. INTRODUCTION

Right from the start of the Industrial Revolution, the global community has continued to witness an unprecedented increase in temperature all over the world [1, 2]. In 2021, a record-breaking level of approximately 36.3 Gt of CO₂ emissions from the combustion of fossil fuel and industrial activities was recorded globally [3]. Greenhouse gas emissions caused by humans were reported by the Intergovernmental Panel on Climate Change (IPCC), and the abovementioned emission results in an increase of 1.1°C above pre-industrial temperatures, while a 1.5°C rise has been

forecast for the early period in the 2030s [4]. As a drawback, the global climate system is changing with increases in flooding, drought, rise in sea level and severe heatwaves [5, 6]

The poor air quality that causes severe damage to the environment and human health also affected cities like Los Angeles and Beijing, the wealthiest and most highly regulated jurisdictions around the world. The main cause of the aforementioned predicament (poor air quality) is the combustion of fossil fuel, with the contribution of over 80% of total emissions from greenhouse gases [7, 8, & 9]. However, as contemporary civilization's living standards improve, especially in areas like industrial, commercial, transportation, and residential reasons, there is a gradual increase in the global energy hunger despite consistent increases in energy conversion efficiency. In light of this, scientists, environmentalists, and policymakers worldwide have been striving to develop and implement workable (and economical) alternatives and policies for the eradication of the negative effects of the conversion and use of fossil fuels around the world [10].

To tackle the above challenges, the focus has shifted by researchers around the world towards renewable energy source development, while among different sources of renewable energy; biofuels have displayed significant potentiality towards meeting the current energy demand. However, microalgae have currently emerged as promising feedstock in the production of biodiesel [11]. The photosynthesis-driven factories that employed light in carbon dioxide transformation into carbohydrates, proteins, lipids, vitamins, and pigments are considered microalgae [11]. Despite the aforementioned advantages, various research works targeted towards microalgae-based biodiesel commercialisation face important problems that are not limited to the high cultivation, harvesting, and lipid extraction costs but also include scaling up to meet market demands [12]. The success of microalgae biodiesel's full potential in commercial activities rests

on sustainable practices like cost reduction in production systems and improved cultivation systems [13].

High investment costs, low biomass and lipid productivity, and high downstream processing costs are major barriers to the economic feasibility of large-scale commercial cultivation of microalgae for biodiesel production [14, 15]. Another issue facing microalgae-based biodiesel is the construction of large-scale production facilities to satisfy market demand [16]. The high expenses associated with the production, harvesting, and extraction of biomass and lipids are another problem; therefore, efforts should be directed toward cost reduction and efficiency enhancement in order for biodiesel from microalgae sources to compete with and surpass fossil fuels [17].

Contamination risks, lack of online monitoring, unpredictable weather situations, and control of process parameters are problems facing open cultivation systems, while closed systems are associated with high capital costs, high biomass productivity, and low risk of contamination [18]. For the production of biodiesel that satisfies regulatory requirements and to ensure low-cost, high-quality photobioreactors, the current technologies used in microalgae-based biodiesel must be urgently modified. To produce biodiesel that is profitable, several technological problems must be resolved [15]. According to Zhao et al. [19], a photobioreactor (PBR) is a closed tank that receives energy from electric lights. The production of biodiesel on a large scale in closed photobioreactor systems is marked by difficulties with capital costs related to acquisitions, maintenance problems, and operating processes [20, 21]. Therefore, developing and building inexpensive, efficient, and functional photobioreactors with low construction and operating costs is essential for large-scale industrial microalgae cultivation [22].

Due to their ability to respond to changes in local environmental circumstances, microalgae from local habitats have been demonstrated in earlier studies to be robust and competitive. These qualities are essential for effective large-scale production [23, 24]. One tactic that can greatly lower costs and improve an environment is the production of robust strains of microalgae that have the capacity to flourish in various environmental conditions [25, 26, 27]. Lim and Schenk's [28] research also claimed that strain selection and growth and condition optimisation contributed to a high lipid output. Therefore, the present work considered the green microalgae strain, *Crucigenia* sp. OW2, isolated from a local freshwater fish farm in Owode Ede, Osun State, Nigeria.

To increase the microalgae's economic viability for the production of biodiesel, productivities with relation to biomass and lipid production are crucial factors to optimize [29]. Optimizing environmental factors and controlling environmental culture conditions are effective ways to boost lipid production in microalgae and lower the cost of microalgae-based biodiesel production [30, 31, 32, 33, 34]. Environmental factors like temperature, light intensity, carbon and nitrogen, pH levels, and nutrient availability have a big impact on microalgae growth and lipid production [35, 36, 37, 38]. Optimizing growth media and culture conditions is essential to ensuring the provision of sustainable, economical biomass production [39]. Thus, by optimizing different production and nutrient parameters, the current study aims to increase biomass and lipid production in *Crucigenia* sp. OW2.

By controlling every aspect of the culture environment, microalgae growing in a closed photobioreactor system ensure the highest possible production yield [40]. The ideal photobioreactor for microalgae strains should be chosen based on performance, cost-effectiveness, and output, even though they are divided into tubular or columnar, flat-plate, and stirred-tank types based on how mixing is achieved [24]. Photobioreactors should have low mutual shading, a

transparent surface, uniform illumination, and quick mass transfer of carbon dioxide and oxygen, in addition to achieving higher growth and biomass production [41]. In light of this, the main focus of this work is the design and construction of a 50 L tubular-shaped photobioreactor for the upscale production of biomass and lipids by *Crucigenia* sp. OW2. The reactor is constructed from inexpensive but high-quality materials, has transparent glass, and is uniformly lit by LED bulbs. Due to their long lifespan, high luminous efficacy (between 25 and 64 lm/W), and narrow emission bandwidth that coincides with photosynthetically active radiation, LEDs are a promising choice for photobioreactors [42]. Recent research by Amaral et al. [41] and Winkler et al. [43] validated the usefulness of LEDs in photobioreactor internal illumination and attested to their potential for cultivation and biocatalysis.

This study's primary goal is to address the following issues: low biomass and lipid production; problems with microalgae cultivation and procurement costs; operation of the bioreactor by selecting *Crucigenia* sp. OW2 from the local environment; optimization of various cultivating and nutritional parameters; selection of a closed system for the growth of the microalgae strain; and design and construction of a 50 L cylindrical glass-stainless vessel photobioreactor. The major novelty of this work is the designed and fabricated photobioreactor. The primary novelty of the developed photobioreactor lies in the integration of hybrid mixing, internal LED illumination, and modular stainless-steel construction within a medium-scale cultivation platform. Although individual elements of these design features have been explored in previous photobioreactor studies, their combined implementation in a locally fabricated 50-L system provides a unique approach for improving microalgae cultivation efficiency.

Additionally, the reactor was built with a focus on process intensification and cost-effectiveness, which addressed one of the main obstacles to the production of microalgal biofuel on a large scale:

the high capital and operating costs of traditional photobioreactor technologies [44]. The presented system offers a flexible platform for examining microalgal biomass productivity, lipid accumulation, and biodiesel generation under scaled cultivation circumstances by combining regulated illumination, effective mixing, and sturdy reactor construction.

2. METHODOLOGY

2.1. Microorganisms, culture media and aeration system

Crucigenia sp.OW₂ was one of the thirty-three microalgae genera that were identified using a light microscope from water samples collected from a freshwater fish farm in Osun State at Owode-Ede, which is located between latitude and longitude 7.7127 and 4.4954 degrees. Among the twelve strains that were effectively isolated is OW2 [45]. Morphological study under a microscope revealed cells with chloroplasts, an oval or triangular form, and a light green tint. The strain is called *Crucigenia* sp. OW2, as shown by the colony's appearance, which includes four connected cells packed together and an empty space in the middle [45]. The isolate was maintained in Bold's Basal Medium (BBM) with a photoperiod of a 16/8 h light/dark cycle and continuous lighting at 850 lux for three weeks.

2.2 Optimisation process

2.2.1 Optimisation of environmental and culture parameters

The pH range of 5 to 9 used in this study is the modification of the pH of 5 to 8 used by Paramasivam et al. [46]. This work modifies the 10–30°C (10, 20, and 30°C) temperature range used in the study conducted by Can et al. [47] to the 20, 25, 30, and 35°C temperature ranges. In

the study conducted by Putri et al. [48], the light intensity of 2000, 2500, 3000, and 35000 lux is changed to the range between 1000 and 4000 lux. Putri et al. [48] used photoperiods of 24:0, 20:4, and 18:6 hours; they were changed to 8:16, 12:12, 16:8, 20:4, and 24:0 light:dark cycles in this work. The agitation rates between 50 and 250 rpm (50, 100, 150, 200, and 250 rpm) were substituted for the 50, 100, 150, and 200 rpm used in the work done by Adekanmi et al. [49]. Using six different culture media (Bold Basal Medium (BBM), CHU-10, Fogg's, BG-11, CFTRI, and Zarrouk's Medium (ZM)), this investigation adhered to the approach of Dixit et al. [50].

2.2. Nutrient Optimisation

Sodium chloride concentrations of 10, 20, 30, and 50 PSU were used in this study, as they were in the research reported by Adekanmi et al. [49]. In this investigation, glucose, ethanol, sucrose, fructose, and acetate were substituted for the carbon sources of glucose, ethanol, and glycerol utilized in the research conducted by Ogbonna et al. [51]. The current investigation used glucose values of 5, 10, 15, 20, 25, and 30 g/L instead of the 5, 10, 15, and 20 g/L used by Ogbonna et al. [51]. The nitrogen sources (sodium nitrate, potassium nitrate, yeast extract, peptone, and urea) used in the work carried out by Adekanmi et al. [49] were used in the current work. The sodium nitrate concentrations (0.40 g/L, 0.80 g/L, 1.5 g/L, and 3 g/L) employed in the work conducted by Can [47] were modified (0.8, 1.5, 3, 4.5, and 6 g/L) in the current work. Phosphorus concentrations (KH_2PO_4) between 0.33 and 1.6 g/L (0.33, 0.43, 0.82, 1.2, and 1.6 g/L) are examined in this study, according to Montoya-Vallejo et al. [52]. The concentrations (0.01, 0.02, 0.03, 0.04, 0.045, and 0.05 g/L) used by Chandra et al. [53] were changed to 0.01, 0.02, 0.03, 0.04, 0.045, 0.05 g/L, 0.06 g/L, and 0.07 g/L of iron, respectively.

2.3. Photobioreactor

2.3.1. Photobioreactor Design and Configuration

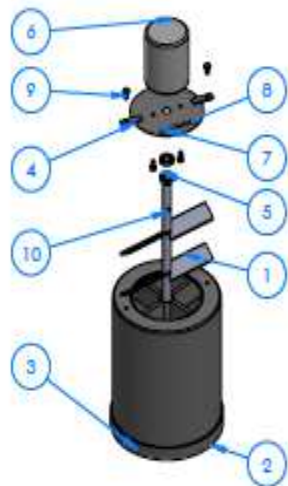
The microalgae strain (*Crucigenia sp.*) was cultivated in a vertical cylindrical-shaped, locally fabricated photobioreactor (Figure 1) to provide controlled culture conditions and an aseptic environment. This reactor was made from polished stainless steel (SS304/316) that ensures resistance to corrosion, simple and stress-free sterilization, illumination suitability, and agitation protocols [54, 55]. The geometry of this photobioreactor was selected carefully to ensure the balance of light penetration, hydrodynamic performance, and scalability as required by the stipulated principle for the design of photobioreactors recently reported by Posten [56] and Sathinathan et al. [57].

The height to diameter (H/D) ratio of the reactor was roughly 2.3, with an internal diameter of 0.30 m and an internal height of 0.70 m (Figure 1). This ratio is within the suggested ideal range (2–3) for stirred photobioreactors, where effective axial mixing reduces dead zones and successfully improves gas and liquid transport without requiring excessive energy input. The reactor has a total volume of 50 L, of which 42 L are used for culture cultivation and the remaining 8 L are headspace (16% of the total volume) for gaseous exchange and pressure moderation [58, 55]. In comparison to rectangular vessels, the cylindrical vertical shape is chosen to ensure efficient circulation, reduce stagnant zones, and improve the useful surface-area-to-volume ratio.

This reactor incorporates a hybrid mixing system, which consists of a mechanical impeller (a low shear) at the bottom of the vessel and a gas diffuser (a fine pore) for aeration and CO₂ supply (Figure 1). The aforementioned system enhances photosynthetic efficiency and biomass production by promoting mass transfer and ensuring uniformity in nutrient distribution while exposing the cells to alternating cycles of light and dark photoperiods. This reactor does not include

baffles; however, the careful selection of a low-speed impeller addresses concerns related to shear stress and vortex formation, while achieving homogeneous mixing without excessive shear that could cause cellular damage, thanks to the height-to-diameter ratio of the photobioreactor. To promote mixing of inoculum and production medium, enhance mass transfer, and ensure uniform distribution of cells throughout the photobioreactor's illuminated zone, a mechanical impeller that is driven by an adjustable-speed motor is centrally fitted. At the bottom of the reactor is the fitted gas diffuser that improves air dispersion and fine bubble CO₂ while also enhancing carbon dioxide dissolution and promoting removal of oxygen [58].

The LED rods are positioned internally around the reactor vessel to serve as source of the illumination to the culture. The energy efficiency, low output of heat and emission within the range of 400–700 nm photosynthetically active spectrums are the reason behind the choice of LEDs. The arrangement of inside LEDs minimizes light attenuation and self-shading, allowing for the regulation of light intensity at different depths within the culture. The reactor's LED light source distributes photosynthetically active radiation (PAR) throughout the culture volume. According to Legrand et al. [59] and Shaikh et al. [55], the reactor's internal LED light source has been found to maximize biomass productivity while promoting an equitable distribution of photons. The reactor's cover is bolted to maintain aseptic conditions; CO₂ injection ports are attached to guarantee carbon supply control; and a sampling port is used to remove sterile sample cultures. Headspace permits oxygen to build up and escape, preventing oxygen inhibition and promoting a healthy aeration system.



ITEM NO.	PART NUMBER	QTY.
1	Impeller.	1
2	Reactor base	1
3	Reactor vessel	1
4	Reactor vessel cover	1
5	Bearing	2
6	Motor.	1
7	Gas diffuser	1
8	LED Bulbs	4
9	Bolt	4
10	CO2 Port	1

Figure 1: 50L vertical cylindrical-shaped tubular stainless steel photobioreactor

2.3.2. Preparation of Culture Media

Standard procedures were followed in the preparation of a sterile BG-11 growth medium. The pH was brought to 8 while the medium was dissolved in deionized water to guarantee the best possible nutrient availability. The preparation and sterilization of the medium were done in accordance with the usual procedures for cultivation in a closed photobioreactor [60]. Prior to rinsing with sterile deionized water, the reactor was first chemically sterilized in situ using diluted hypochlorite solution [60]. Before being aseptically transferred into the photobioreactor, the vast production volume was sterilized in an autoclave. The impeller, gas diffuser, and lid (all reactor components) were cleaned using a laboratory-grade detergent. They were then disinfected with 70% ethanol and rinsed both before and after each experiment to ensure sterility and sustainability. Aeration was

accomplished using filtered air and CO₂ (0.22 µm) to further eliminate contaminants. The medium was aseptically moved into the reactor vessel after being sterilized to the predefined working volume of 42 liters. The medium was aseptically placed into the reactor vessel to a predefined working volume of 42 liters after it had been sterilized. The reactor cover was fastened with bolted fittings to guarantee an airtight and sterile enclosure.

2.3.3 Microalgal Strain and Inoculum Preparation

Crucigenia sp. stock cultures were subcultured every two weeks, and the strain was also kept in the exponential growth phase, which is crucial for shortening the time it takes for cells to adapt after being inoculated into bioreactors [58, 59]. The culture of *Crucigenia* sp. was harvested at an optical density (OD₆₈₀) of 0.8–1.0 for inoculation because this is the range that is usually employed for active proliferation of cells while also reducing the possible lag phase in a photobioreactor [58, 59]. The culture working volume of 10% was used as the standard for the inoculum volume, thereby resulting in 4.2 liters of inoculum for a reactor volume of 42 liters. To ensure balance in biomass production and stability in culture dynamics, the above fraction of inoculum has been generally deployed in photobioreactor experiments [55, 59]. The total working volume (culture volume) in the photobioreactor was restored to 42 liters after inoculation through the addition of the required sterile medium of BG-11.

2.3.4 Reactor Operation and Environmental Control

Following inoculation, the reactor's operation starts at a modest impeller speed of 150 rpm to activate mechanical agitation; this reduces shear stress and maintains uniform mixing. This speed gradually increased as the culture adjusted to the reactor environment, guaranteeing that shear stress was within the necessary bounds for cell health [58]. In combination with the diffusion of

the gas, a low-shear impeller ensures circulation without inducing a vortex causing damage of the cells. There was simultaneous initiation of aeration at 0.5–1.0 vvm, where filtered air that was enriched with CO₂ (1–5%) was supplied and delivered by a diffuser (fine pore) for stabilization of pH and functional gaseous exchange. Through the diffuser, fine bubble aeration enhances gas-liquid mass transfer and also promotes effective uptake of carbon in the culture medium [59, 55]. Light was delivered to the reactor using an LED system at an intensity of 3500 lux. According to Shaikh et al. [55] and Legrand et al. [59], regulated lighting improves photosynthesis and biomass production efficiency while preventing photoinhibition. The ideal photoperiod of a 24-hour light and dark cycle was maintained for the production of biomass and lipids by *Crucigenia sp.* Temperature and pH were both tracked throughout the reactor's cultivation time. The ideal culture pH of 8 was regulated by administering CO₂, and the temperature was kept at 25°C using an external thermostatic control.

2.3.5 Monitoring and Sampling Procedures

Samples were collected in aseptic settings every 24 hours. To lower the danger of contamination, culture samples (50 mL) were removed using a special sampling port.

2.3.6 Cost-Effectiveness and Materials

LED rods, bolts, and gaskets, as well as a stainless-steel tank, impeller, motor, and gas diffuser, were included in the bill of materials (BOM) (Table 1). Commercially available 50-L PBRs were compared to component costs and the total fabrication cost (~\$1,100), demonstrating the possibility for cost savings through modular design, local production, and energy-efficient LEDs.

Table 1: Photobioreactor Bill of Materials

S/ N	Component	Quantity	Unit Cost (\$)	Total cost (\$)
1	Stainless steel vessel	1	600	600
2	Impeller and motor	1	250	250
3	Gas diffuser	1	50	50
4	LED rods (internal)	6	30	180
5	Bolts and gaskets	1 set	20	20
Total Fabrication				1.100

2.3.7 Cleaning, Sterilization, and Contamination Control

To prevent contamination and ensure reproducibility of the results in closed photobioreactor systems, proper cleaning and sterilization before and after each experiment are important [60]..

All components (vessel interior, agitator shaft and impeller, gas diffuser, and sampling ports) of the reactor were disassembled before the experiment and cleaned using laboratory-grade detergent.

After rinsing with water (deionized) under a high-temperature solution of sodium hypochlorite

(0.5–1%), it was used to sterilize the reactor and left in contact for a period of twenty minutes. To remove residual disinfectant, sterile deionized water was used to flush the entire reactor system. At the end of the experiment run, the reactor was completely drained and rinsed to prevent possible deposition of biomass. This was followed by the disassembling of all reactor components, scrubbed and finally cleaned through preliminary sterilization procedures. Before final rinsing and storage, detachable components, such as the gas diffuser, were further soaked in diluted acid to eliminate any potential mineral deposits. To maximize the experiment's integrity and avoid any contamination, the aforementioned cleaning and sterilizing techniques are consistent with established standards for culture in photobioreactors [60].

2.3.8 Safety, Sterilization, and Operational Considerations

The use of a stainless-steel reactor vessel lowers the chance of breakage, and lines providing gas, including check valves and regulators to prevent overpressure, are insulated along with electrical components like motors and LEDs. Aseptic inoculation, filtered gas supply, and sterilization procedures maintain the culture integrity in the reactor. Bolted modular designs provide safe and repeatable operation while facilitating simpler disassembly during cleaning and maintenance procedures.

2.4 Techno-Economic Analysis (TEA)

To evaluate the economic feasibility of the hybrid-mixing-fabricated photobioreactor for the production of biodiesel, a preliminary techno-economic analysis was carried out. The capital expenditure (CAPEX) and operating expenditure (OPEX) were considered. The stainless-steel vessel, internal LED system, mixing unit, and gas delivery components were the bill of materials (BOM) for the fabrication of the reactor (~\$1,100) and were used for estimation of CAPEX. For

the estimation of OPEX, nutrient costs, and labor and energy consumption by LED illumination, agitation, and aeration were considered.

Due to the lack of direct power measurements, the energy consumption figures were calculated using reported normal ranges for laboratory-scale photobioreactors in the literature. The energy requirements for LED lighting were previously reported to be between 50 and 100 W, agitation systems to be between 50 and 150 W, and aeration units to be within comparable ranges for small-scale systems [61, 62].

In techno-economic analyses of microalgae cultivation systems, the above values have been widely utilized and are considered ideal for preliminary evaluation [63, 64]. Therefore, this analysis showcases an indicative evaluation of economic performance rather than an absolute prediction of cost.

Energy consumption was calculated using:

LED power rating $\times$ operating hours

Motor power $\times$ agitation time

Air compressor energy demand

The cost of biodiesel production (CBP, \$/L) was estimated as:

$$CBP = (\text{Annualized CAPEX} + \text{OPEX}) / \text{Annual biodiesel output}$$

2.5 Sensitivity Analysis

This was carried out for evaluation of the impact of important process and economic factors on the production of biodiesel. The parameters selected include biomass productivity (g/L), lipid content, energy cost (\$/kWh), and reactor scale (production volume). Based on normal variability found in microalgal cultivation systems and techno-economic studies, the sensitivity ranges (± 20 –50%) were chosen. To determine the main cost drivers, the effect on the cost of producing biodiesel (\$/L) was assessed. This strategy is in line with common techno-economic techniques applied to assessments of biofuel systems [65, 66], which typically involve evaluating cost-effectiveness, resource allocation, and potential environmental impacts.

2.6 Sustainability Metrics Assessment

The key environmental indicators like carbon dioxide (CO₂) fixation rate, energy efficiency (biomass produced per unit energy input), and lipid productivity per unit resource input, and waste minimization and material recyclability were used for the evaluation of sustainability performance. CO₂ fixation was estimated based on biomass productivity, assuming ~1.83 kg CO₂ fixed per kg biomass. Energy efficiency was calculated as biomass yield per kWh consumed.

2.7. Scale-Up Considerations

Geometric, hydrodynamic, and operational parameters of the reactor were considered for assessment of scale-up potential. Key criteria included: Surface-area-to-volume ratio; Light penetration depth, mixing efficiency (hybrid system performance), and gas–liquid mass transfer capability. By comparing design characteristics with accepted photobioreactor scale-up principles, the system's scalability was assessed. The viability of the cylindrical geometry and modular architecture for pilot- and industrial-scale applications was examined.

2.8. Growth Parameters

2.8.1 Biomass Concentration and Productivity

A 10 mL culture of *Crucigenia* sp. was placed on a Whatman GF/C microfiber filter with a 4.7 mm diameter in order to determine the dry cell weight. The biomass was washed twice with distilled water before being dried for 24 hours at 105°C in order to calculate the dry cell weight (g). The biomass concentration (g/L) and biomass productivity (PBIOMASS, g/L/day) were calculated using Niels et al.'s approach [67].

2.8.2 Total lipid productivity

This was conducted by using the method described by Bligh and Dyer [68], where a 3 mL mixture of methanol and chloroform in a 2 to 1 ratio was added to a known biomass of *Crucigenia* sp. and later incubated for a period of 24 h at room temperature. This was followed by repeated centrifugation in a centrifuge at 1078 rpm for 10 minutes at each cycle, while 2 mL of potassium chloride (1%) was added to the supernatant obtained, resulting in the formation of two layers. The upper layer was decanted while the lower layer contains lipid and is carefully removed and weighed. The equations (1 &2) below were used for calculating the lipid content (LC) and lipid productivity (LP).

$$\text{Lipid Content (g L}^{-1}\text{)} = (W_2 - W_1) / B \text{ ----- (1)}$$

Where, W1 = Empty beaker weight

W 2 = weight of beaker contains dry lipid

B = Dry Biomass weight

Lipid Productivity ($\text{g L}^{-1} \text{ day}^{-1}$) = Lipid content X Biomass productivity ----- (2)

2.9. Transesterification of lipids

This was accomplished by modifying the method described by Zahran and Tawfeuk [69]. It involves combining 1.0 mL of n-hexane with 15 mg of lipid. Prior to this, 0.4 mol of sodium methoxide (1.0 mL) was added. The mixtures were vortexed for 30 minutes and then left to settle for 15 minutes. The upper part containing Fatty Acid Methyl Esters (FAMES) was recovered using a disposable nylon syringe filter (0.2 μm pore, 13 mm diameter; Cronus, UK).

2.10. FAME characterization

The FAME composition was determined using a GC/MS. A GC/MS with an Rtx5 column (30 m $\times$ 320 μm $\times$ 1.0 μm film thickness) was filled with one microliter of FAME extract (Supelco, USA). With a split of 100, standard split injection was employed. The injection temperature was set at 180 $^{\circ}\text{C}$, the column oven temperature was set at 140 $^{\circ}\text{C}$, and it was raised to 250 $^{\circ}\text{C}$ at a rate of 4 $^{\circ}\text{C}$ per minute. The detector was set at 220 $^{\circ}\text{C}$ with a carrier gas flow rate of 50 mL/min. The standard FAME combination utilized was Supelco C14:0 to C22:0. The FAME was quantified using peak areas.

2.11 Fuel properties

Biodiesel properties such as cetane number (CN), iodine value (IV), cold filter pour point (CFPP), kinematic viscosity (ρ), density (ρ), higher heating value (HHV), long chain saturated factor

(LCSF), degree of unsaturation (DU), and saponification value (SF) were calculated using the FAME composition of the produced biodiesel. CN, CFPP and LCSF [70, 71] and SV, IV, DU, v , ρ and HHV were calculated as in literature [72].

2.13 Statistical Analysis

All experiments were performed in triplicate. Data are presented as mean $\pm$ SD. Correlation and regression analyses were conducted using all experimental replicates to maximize statistical power. One-way ANOVA confirmed that all investigated parameters Pearson's correlation coefficient was used to assess the association between biomass concentration, lipid content and biodiesel produced, while simple linear regression was applied to evaluate the predictive relationship between these variables.

3. Result and Discussion

3.1 Culture and Environmental parameters

The growth and lipid accumulation of *Crucigenia* sp. OW2 were significantly influenced by variations in environmental and culture conditions. Statistical evaluation using one-way ANOVA confirmed that all investigated parameters exerted a highly significant effect ($p < 0.0001$) on both biomass and lipid content, indicating that these variables are critical determinants of microalgal productivity.

3.1.1. pH

The optimal pH for both biomass and lipid production was 8, yielding 5.52 ± 0.07 g/L biomass and $34.57 \pm 0.07\%$ lipid content (Figure 2). The results ($F = 1089.951$ for biomass; $F = 3489.144$ for

lipid) of ANOVA confirm pH has a significant impact on metabolic processes, especially assimilation of carbon (Table 2 & 3). The present work conforms to the works conducted by Priya et al. [73] and Almutairi et al. [74] on *Chlorella vulgaris*, and *Tisochrysis lutea*, where higher biomass and lipid content were recorded at a pH of 8.

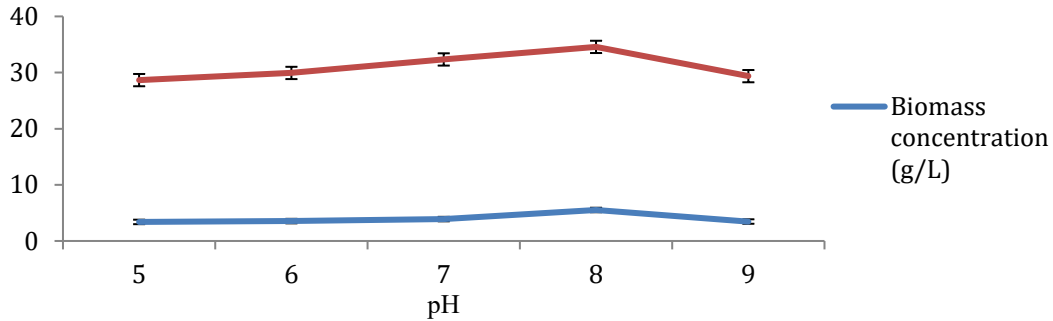


Figure 2: Effects of pH on biomass and lipid production

3.1.2. Temperature

Microalgal growth was strongly impacted by temperature; the best biomass (5.64 ± 0.18 g/L) and lipid content ($34.47 \pm 0.10\%$) were found at 30°C (Figure 3). The results (Tables 2 and 3) obtained using ANOVA ($F = 344.859$ for biomass; $F = 3717.312$ for lipid) revealed that temperature has a significant but relatively mild effect on biomass with a greater impact on lipid production. The current study's findings are connected to studies by Converti et al. [75] and Renaud et al. [76], which found that 30°C was the ideal temperature for biomass and lipids.

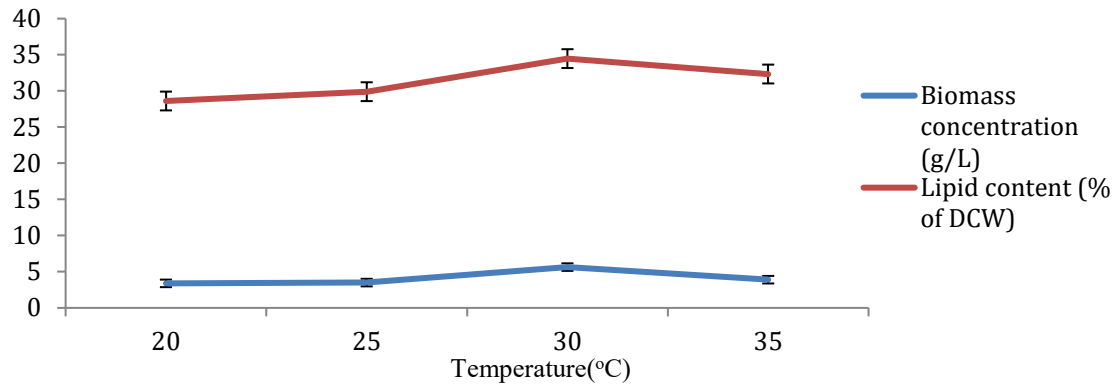


Figure 3: Effects of temperature on biomass and lipid production

3.1.3. Light intensity

The biomass (5.98 ± 0.03 g/L) and lipid content ($35.07 \pm 0.07\%$) were positively impacted by light intensity, with the highest values noted at 3500 lux (Figure 4). This shows that photosynthetic activity was impacted up to a point of saturation with an increase in photon flux. The results of ANOVA revealed that light intensity is a major determinant of photosynthetic efficiency with $F = 2988.081$ (Table 2) and $F = 10604.540$ (Table 3) for biomass and lipid content. A similar result was found in the work carried out by Mohammadi et al. [77], where optimal biomass and lipid were recorded at 3500 lux.

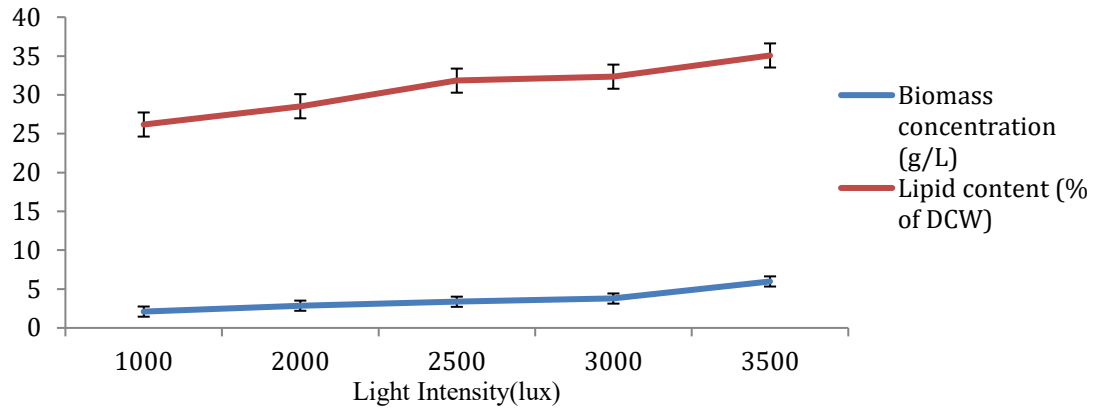


Figure 4: Effects of light intensity on biomass and lipid production

3.1.4. Photoperiod

The best biomass (5.85 ± 0.04 g/L) and lipid content ($34.47 \pm 0.06\%$) were observed at continuous illumination (24:0 h), indicating that prolonged light exposure improves fixation of carbon and lipid synthesis (Figure 5). The results showed that constant exposure to light produced the best productivity, indicating that continuous photosynthetic activity improved biomass and lipid accumulation. ANOVA analysis results indicate that biomass and lipid had $F = 2460.556$ (Table 2) and $F = 837.412$ (Table 3), respectively; thus, photoperiod also had significant effects ($p < 0.0001$). The results of this study are consistent with those of a study by Viena et al. [78], which found that increased biomass and lipid content were achieved with a photoperiod of 24:0 hours.

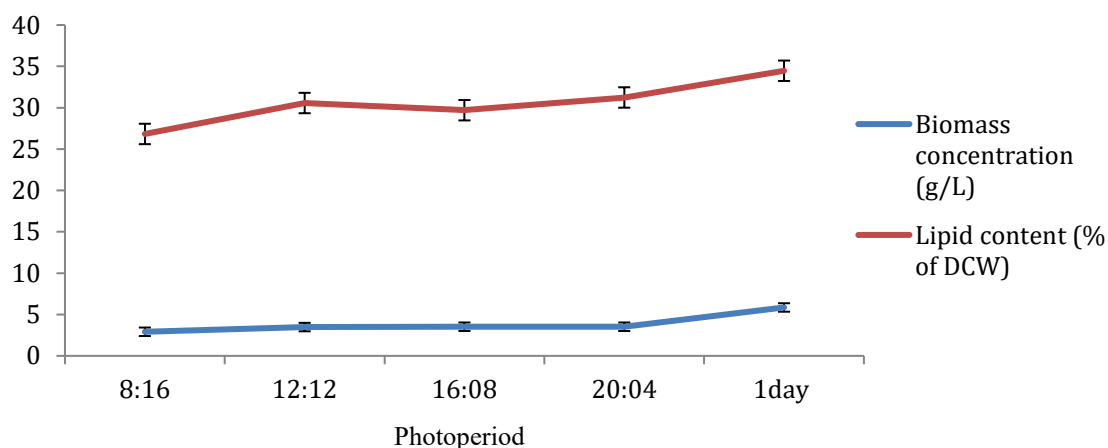


Figure 5: Effects of photoperiod on biomass and lipid production

3.1.5. Agitation speed

The culture performance was impacted significantly, with the highest biomass (6.30 ± 0.06 g/L) and lipid content ($36.44 \pm 0.05\%$) noted at an agitation speed of 200 rpm. This finding indicates that high agitation at 250 rpm reduces productivity due to shear stress, while insufficient mixing and mass transfer lead to low productivity at lower agitation speeds; however, adequate mixing improves mass transfer, light exposure, and nutrient distribution. The finding enumerates the essence of optimal hydrodynamic conditions in enhancement of nutrient distribution, gaseous exchange, and light exposure while mechanical damage to the cells is avoided. The ANOVA findings revealed the exceptionally high F-values ($F = 2928.151$ for biomass; $F = 10262.122$ for lipid), indicating that agitation, a crucial operational parameter, has an impact on reactor performance (Table 2 &3).

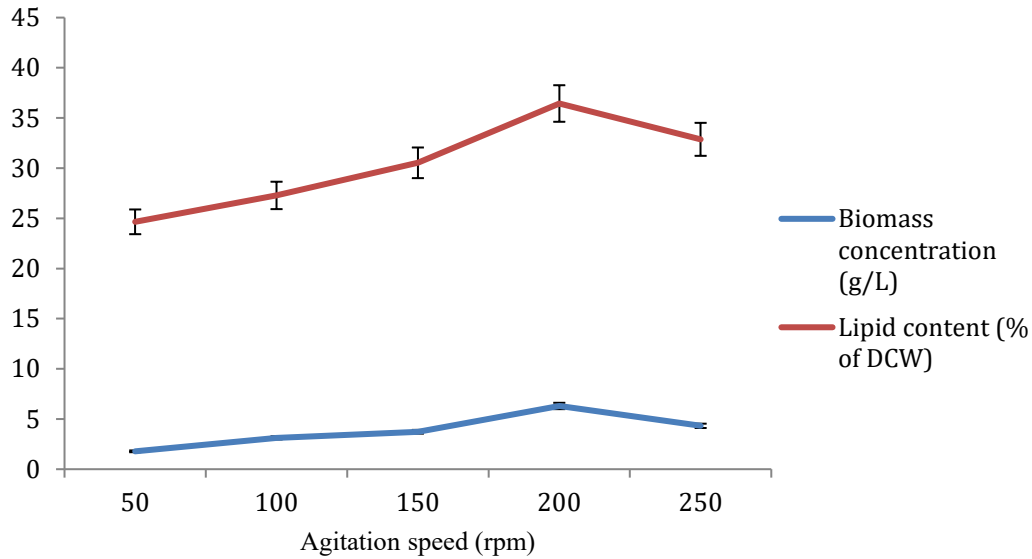


Figure 6: Effects of agitation speed on biomass and lipid production

3.1.6 Culture media

BG-11 provided the highest lipid content ($35.98 \pm 0.07\%$) among the tested media, while Zarrouk medium produced the highest biomass (5.13 ± 0.05) [Figure 7]. The results of the ANOVA [$F = 2588.965$ for biomass (Table 2) and $F = 11798.407$ (Table 3) for lipid] demonstrate that medium composition is a critical factor in metabolic control, impacting the pathways involved in growth and lipid synthesis. Variations in the availability of micronutrients and nitrogen, in particular, can be linked to this variance, as nutrient-rich media encourage growth, whereas limited or somewhat balanced media stimulate the buildup of lipids. The nitrate concentration, which is ten times higher in Zarrouk's medium than in BBM [79], is most likely the cause of the highest DCW in Zarrouk's and the lowest in BBM, because carbon is also one of the essential components needed for microalgae growth [80].

In connection with this work, a number of researchers have discovered that microalgal strains grown in BG-11 have the highest lipid content when compared to other growth media; Karpagam [81] reported 24 and 26% for *Coelastrella* sp. M-60 and *Micractinium* sp. M-13; Song et al.[82] reported lipid yield (w/w) of 19.40 & 30.70 for *Chlorella* SDEC-4 and *Scenedesmus* SDEC-9; and Rios et al. [83] found optimal lipid content when using the Guillard F/2 medium. Using BG-11 as the culture medium, Sharma et al. [80] and Gour et al. [84] also reported higher lipid content.

The increased activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a crucial enzyme for the conversion of three phosphoglycerates in microalgae and a substrate for the biosynthesis of carbohydrates and fatty acids, is linked to the higher lipid production observed in BG-11 as microalgal culture media compared to other culture media and related to this study [85]. The availability of iron (Fe) and citric acid in ferric ammonium citrate as additional elements in BG-11 may also be a contributing factor. Citric acid helps with salts that are crucial in preventing the other chemical components of the growth media from precipitating, which increases their abundance in the cells and is essential for increased biomass and lipid production [50].

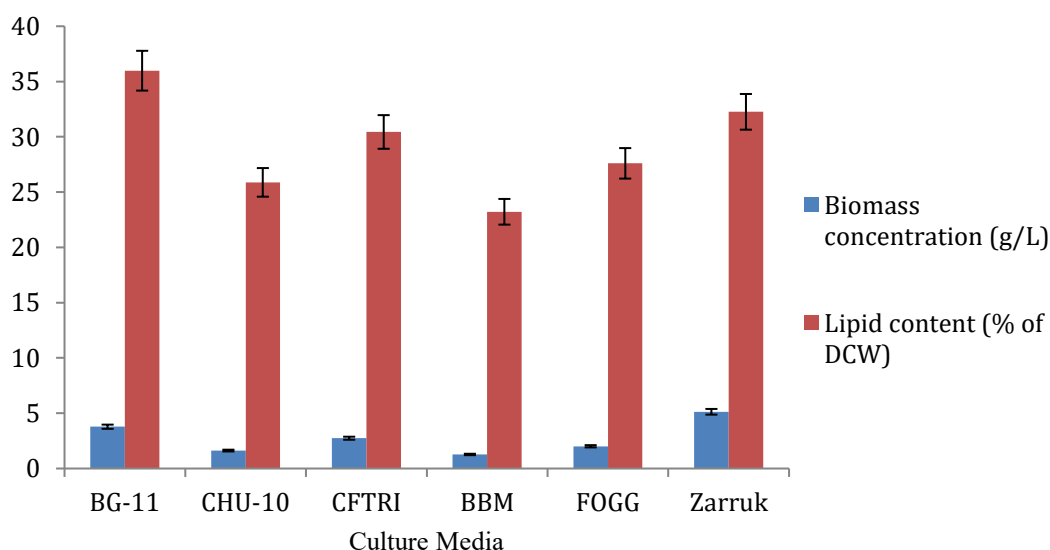


Figure 7: Effects of culture media on biomass and lipid production

3.2. Nutrient Optimisation

3.2.1 Sodium chloride

.The production of biomass was significantly inversely affected by salinity; the maximum biomass (4.83 ± 0.03 g/L) was observed at 10 PSU, and it gradually decreased at increasing salinities. Lipid content, on the other hand, rose gradually with salinity and peaked at 50 PSU at $36.18 \pm 0.01\%$ (Figure 8). This pattern suggests that increased salinity-induced osmotic stress inhibits cellular development while inducing lipid accumulation as a defense mechanism. The activation of stress-response pathways, which divert surplus carbon into neutral lipid synthesis as an adaptation mechanism to preserve cellular homeostasis and membrane integrity, is responsible for the rise in lipid content in saline environments. Salt stress has been shown to increase triacylglycerol accumulation in microalgae [86], which is consistent with similar patterns. Salinity is a statistically significant cause of both growth suppression and lipid induction, according to the ANOVA results ($F = 3325.568$ for biomass and $F = 33797.329$ for lipid content, $p < 0.0001$) (Table 2 &3). The significance of salinity as a stress-regulating parameter is further supported by the high F-values, which show significant variability between salinity levels.

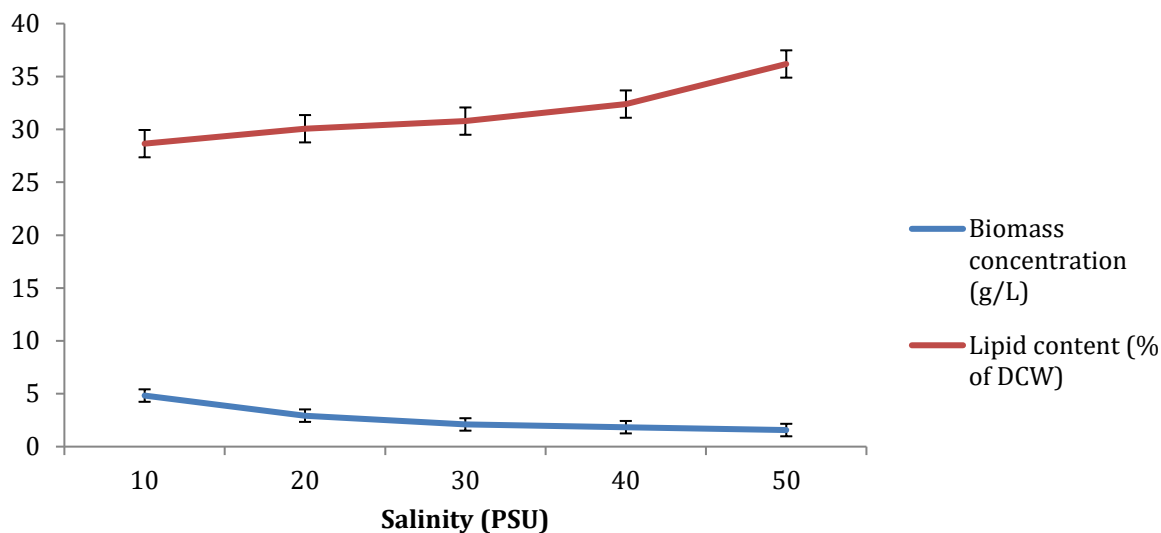


Figure 8: Effects of culture media on biomass and lipid production

3.2.2. Carbon source

The source of carbon had a major impact on the generation of lipids and biomass. Acetate and glycerol were less successful than glucose, which produced the maximum biomass (6.08 ± 0.04 g/L) and lipid content ($35.25 \pm 0.51\%$) (Figure 9). Fructose came in second. Due to its immediate entry into glycolysis, which supplies ATP and acetyl-CoA for the production of fatty acids, glucose performs better than other substances [87]. ANOVA verified the statistical significance of these effects ($F = 4242.900$ for biomass; $F = 500.291$ for lipid content, $p < 0.0001$), suggesting that carbon metabolism is essential for both growth and lipid accumulation (Table 2 & 3).

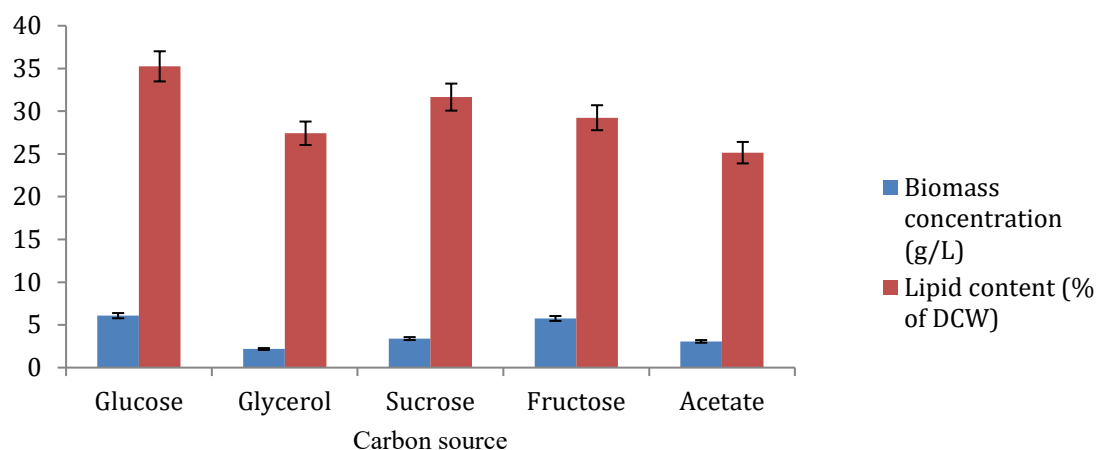


Figure 9: Effects of carbon source on biomass and lipid production

3.2.3. Glucose concentration

The concentration of glucose also demonstrated a dose-dependent effect; biomass and lipid accumulation peaked at 25 g/L (6.08 ± 0.07 g/L and $36.41 \pm 0.07\%$, respectively), after which biomass decreased, indicating osmotic or substrate inhibition (Figure 10). Increased carbon available for lipid production is reflected in the rise in lipid content as glucose concentration rises. ANOVA ($F = 3471.936$ for biomass; $F = 700.299$ for lipid concentration, $p < 0.0001$) [Table 2 & 3] verified the statistical significance of these effects, highlighting the importance of glucose concentration at 25 g/L in both growth and lipid accumulation.

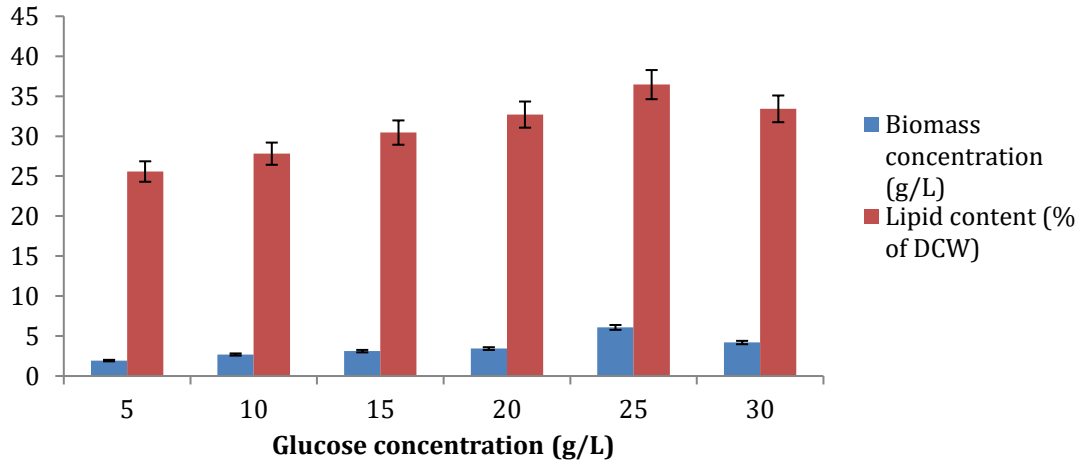


Figure 10: Effects of glucose concentration on biomass and lipid production

3.2.4. Nitrogen source

In comparison to organic nitrogen sources, the results of sodium nitrate (Figure 11) supported the highest biomass (5.40 ± 0.04 g/L) and lipid content ($36.00 \pm 0.05\%$) because it is effectively absorbed through nitrate reductase pathways, which promote cellular development and protein synthesis [88]. Lipid content was highest at the lowest concentration (0.8 g/L), showing nitrogen limitation-induced lipid formation, while biomass peaked at 4.5 g/L (Figure 12), according to variations in sodium nitrate concentration. Microalgae inhibit protein synthesis and reroute carbon flux toward lipid storage, especially triacylglycerols, when they are starved of nitrogen [86, 89]. ANOVA analysis (Table 2 & 3) showed highly significant effects for nitrate concentration ($F = 7091.976$ for biomass; $F = 20778.626$ for lipid) and nitrogen source ($F = 7446.590$ for biomass; $F = 33978.457$ for lipid), indicating that nitrogen availability is a key regulatory factor in microalgal metabolism.

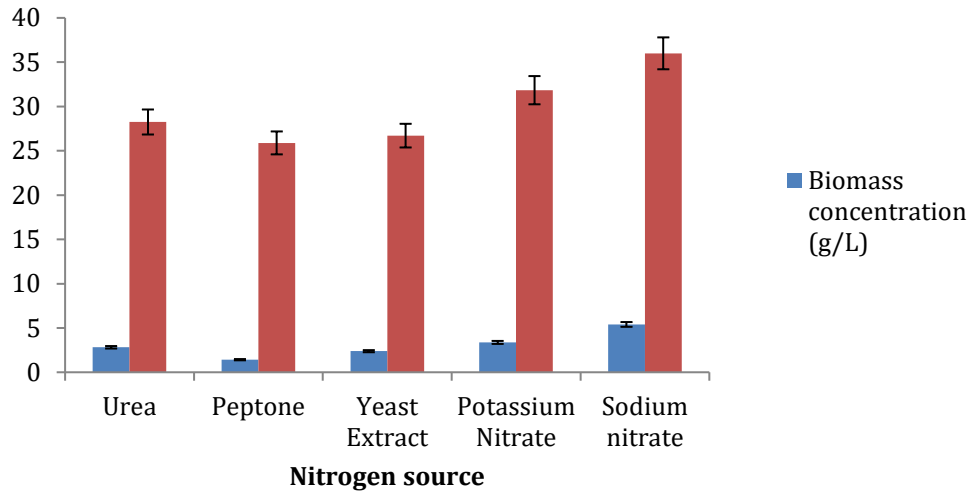


Figure 11: Effects of nitrogen source on biomass and lipid production

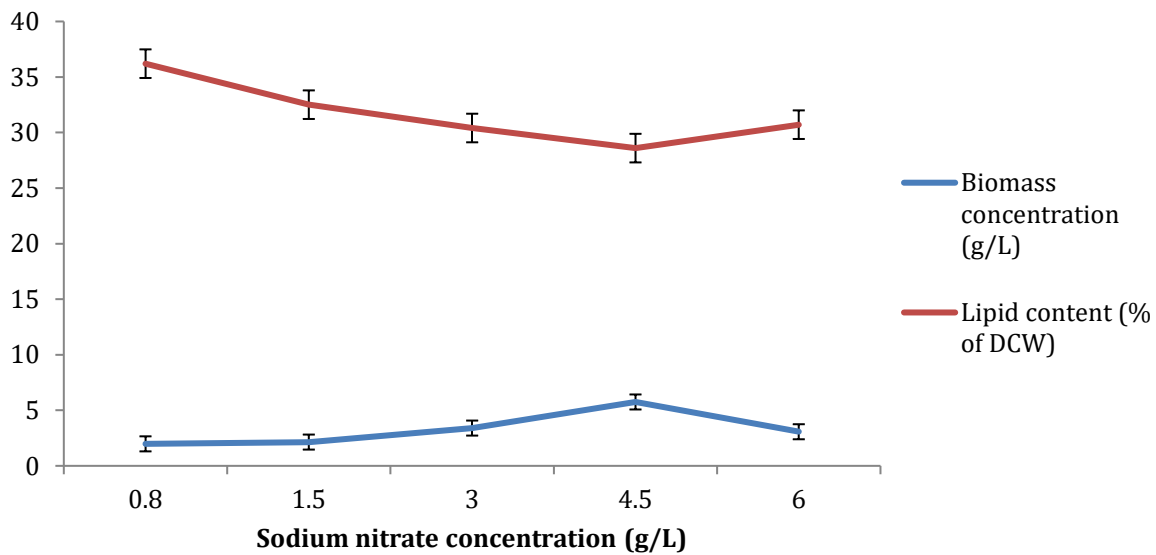


Figure 12: Effects of sodium nitrate concentration on biomass and lipid production

3.2.5. Phosphorus concentration

Lipid content decreased as phosphate availability increased, but biomass increased up to 5.48 ± 0.05 at a concentration of 0.82 g/L (Figure 13). This implies that lipid buildup is triggered by phosphorus constraint. Phosphorus is necessary for the synthesis of ATP and nucleic acids; its

deficiency limits growth and encourages the buildup of storage compounds (Xin et al., 2010). Its function as a metabolic stressor is confirmed by the high lipid content of $38.15 \pm 0.25\%$ at a low phosphate concentration of 0.33 g/L (Figure 13). Phosphate content considerably impacts both reactions; however, its effect on lipid accumulation is relatively stronger, according to ANOVA (Table 2& 3) data ($F = 1472.592$ for biomass; $F = 6820.979$ for lipid, $p < 0.0001$).

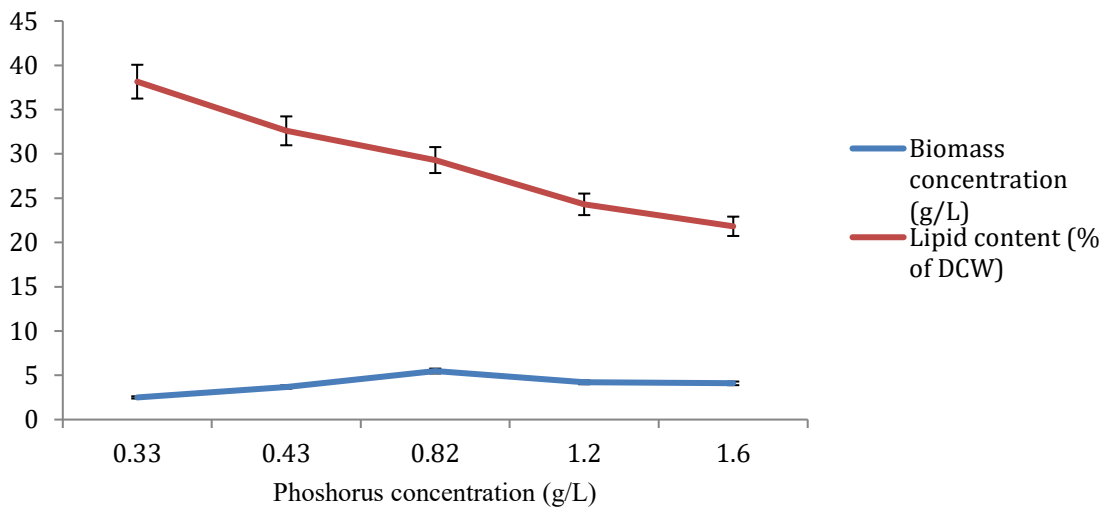


Figure 13: Effects of phosphorus concentration on biomass and lipid production

3.2.6. Iron

Up to an ideal level of 0.05 g/L, iron supplementation increased biomass production (5.31 ± 0.04 g/L), after which a drop was noted. However, at an iron concentration of 0.06 g/L, the lipid content increased marginally to 37.81 ± 0.11 (Figure 14). Cytochromes and ferredoxin are two examples of photosynthetic electron transport systems that depend heavily on iron [90]. Iron promotes growth and photosynthesis at ideal concentrations, but too much iron can cause oxidative stress, which reduces biomass yield. Iron concentration has a considerable impact on metabolic activity,

especially photosynthetic efficiency, according to the ANOVA results ($F = 1963.135$ for biomass and $F = 1547.285$ for lipid, $p < 0.0001$) [Table 2 &3].

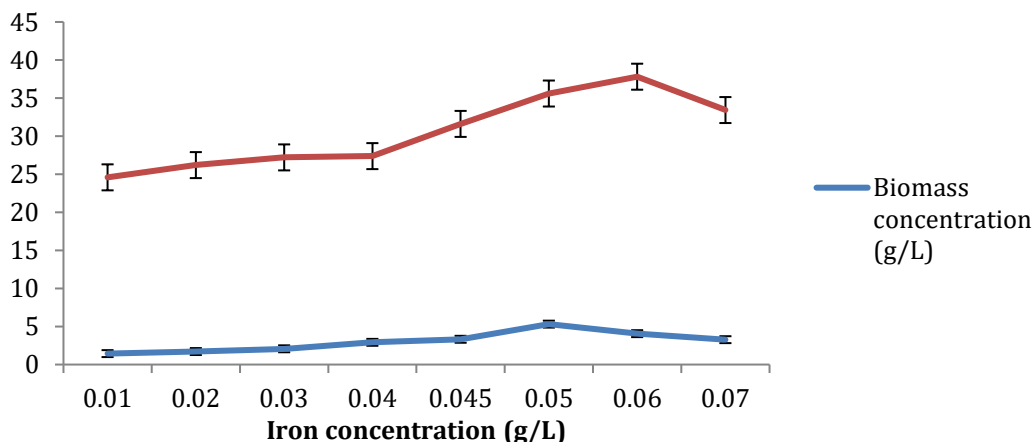


Figure 14: Effects of iron concentration on biomass and lipid production

3.3. Integrated Interpretation and Statistical Validation

One-way ANOVA ($p < 0.0001$ for all variables) confirms the combined results, which show that all examined parameters had a substantial impact on biomass and lipid synthesis. The OFAT approach's efficacy in finding important factors is validated by the consistently high F-values, which show considerable variability between treatment levels.

Table 2: One-way ANOVA for biomass across levels

Variable	df (between, within)	F	p-value	Significant ($p < 0.05$)
Agitation Speed	4, 10	2928.151	< 0.0001	Yes

Carbon Sources	4, 10	4242.900	<0.0001	Yes
Glucose Concentration	5, 12	3471.936	<0.0001	Yes
Growth Media	5, 12	2588.965	<0.0001	Yes
Iron Concentration	7, 16	1963.135	<0.0001	Yes
Light Intensity	4, 10	2988.081	<0.0001	Yes
Nitrogen Sources	4, 10	7446.590	<0.0001	Yes
Phosphate Concentration	4, 10	1472.592	<0.0001	Yes
Photo Period	4, 10	2460.556	<0.0001	Yes
Salinity	4, 10	3325.568	<0.0001	Yes
Sodium Nitrate Conc	4, 10	7091.976	<0.0001	Yes
Temperature	4, 10	344.859	<0.0001	Yes
pH	4, 10	1089.951	<0.0001	Yes

ANOVA results with P-values less than 0.05 indicated that several factors significantly affected biomass yield.

Table 3: One-way ANOVA Results for Lipid Content (%)

Variable	df (between, within)	F-value	p-value	Significant (p<0.05)
Agitation Speed	4, 10	10262.122	<0.0001	Yes
Carbon Sources	4, 10	500.291	<0.0001	Yes
Glucose Concentration	5, 12	700.299	<0.0001	Yes

Growth Media	5, 12	11798.407	<0.0001	Yes
Iron Concentration	7, 16	1547.285	<0.0001	Yes
Light Intensity	4, 10	10604.540	<0.0001	Yes
Nitrogen Sources	4, 10	33978.457	<0.0001	Yes
Phosphate Concentration	4, 10	6820.979	<0.0001	Yes
Photo Period	4, 10	837.412	<0.0001	Yes
Salinity	4, 10	33797.329	<0.0001	Yes
Sodium Nitrate Conc	4, 10	20778.626	<0.0001	Yes
Temperature	4, 10	3717.312	<0.0001	Yes
pH	4, 10	3489.144	<0.0001	Yes

The results of the ANOVA indicate whether changes in each variable resulted in lipid accumulation that was statistically significant. Variables with p-values less than 0.05 were shown to have a significant impact on lipid content.

3.4 Comparative and Critical Evaluation of the Developed 50-L Photobioreactor with Commercial and Existing Systems

3.4.1. Comparison with Conventional Photobioreactor Configurations

3.4.1.1 Tubular Photobioreactors

Commercial microalgae production is dominated by tubular photobioreactors because of their high surface-area-to-volume ratio and scalability. However, several studies have found that biofouling,

oxygen buildup, and uneven light distribution along lengthy flow pathways impair their performance [91]. Long tube light attenuation produces spatial heterogeneity, which lowers photosynthetic efficiency in dense cultures. By removing lengthy flow pathways and greatly lowering light gradients and fouling concerns, the proposed 50-L photobioreactor overcomes these constraints with its compact cylinder shape and internal LED illumination.

3.4.1.2 Flat-Panel Photobioreactors

Flat-panel reactors provide better light utilization because of their large lighted surface area and short optical path length. High biomass productivity and CO₂ fixation efficiency are frequently linked to them [92]. However, high capital expenses, overheating, and structural constraints during scale-up limit their utilization [56]. Through internal lighting, the current reactor replicates the light efficiency of flat-panel systems while retaining a more compact and mechanically sturdy cylindrical shape, which enhances scalability and lowers fabrication complexity.

3.4.1.3 Airlift and Bubble Column Photobioreactors

Airlift and bubble column systems are preferred for their low energy consumption and low shear stress because gas flow drives mixing. But at large biomass densities, these systems frequently have low mixing efficiency, which leads to gradients in light exposure and nutrients [93]. By using a hybrid mixing technique that combines mechanical agitation and aeration to increase circulation and mass transfer, the proposed photobioreactor overcomes this constraint and improves overall reactor performance.

3.4.1.4 Stirred-Tank Photobioreactors

Although stirred-tank systems offer efficient mixing and process control, they are constrained by shear stress brought on by mechanical agitation, which can harm microalgal cells [92]. This

disadvantage is lessened by the proposed system, which uses aeration and a low-shear impeller to ensure sufficient mixing while maintaining cell integrity.

3.4.2. Dedicated Comparison with Commercial Photobioreactors

3.4.2.1 Light Utilization Efficiency

The majority of commercial systems rely on artificial or solar lighting, which restricts light penetration in densely populated areas because of self-shading. Research has indicated that the production of microalgae on a commercial scale is still hindered by light constraint, which lowers biomass output and photosynthetic efficiency [93]. The constructed photobioreactor, on the other hand, uses internal LED illumination, which shortens the optical path length and distributes light more evenly. According to recent research, systems with inside lighting can greatly increase biomass productivity when compared to those with exterior lighting [94].

3.4.2.2 Mixing and Mass Transfer

For mixing, commercial tubular and column photobioreactors frequently use gas flow or circulation pumps, which might not be adequate in situations with significant biomass. Growth may suffer as a result of CO₂ limitation and oxygen buildup [91]. Hybrid mixing (mechanical + aeration) is incorporated into the designed system to boost CO₂ usage efficiency and gas–liquid mass transfer. This strategy is in line with current guidelines that highlight the use of combination mixing techniques to intensify processes [56].

3.4.2.3 Cost and Fabrication

Commercial photobioreactors are frequently linked to expensive fabrication and installation costs, especially for tubular and flat-panel systems that need specialized components and intricate

assembly [92]. The current reactor provides an economical substitute through local production, simplified cylindrical geometry, and the use of easily accessible stainless steel components. This maintains operational efficiency while drastically lowering CAPEX and maintenance expenses.

3.4.2.4 Operational Flexibility and Maintenance

Commercial systems, especially tubular reactors that are prone to fouling, frequently need intricate cleaning techniques. According to Sarker and Kaparaju [91], sterilization and maintenance might be difficult and expensive. A modular bolted design makes it simple to disassemble, clean, and sterilize the constructed reactor. This decreases downtime in between crop cycles and improves operational flexibility.

3.4.2.5 Scalability and Practical Application

Despite being built for large-scale production, commercial photobioreactors frequently lack the flexibility needed for process development and experimental tuning. Laboratory-scale systems, on the other hand, might be difficult to scale. The designed 50-L photobioreactor bridges the gap between industrial applications and laboratory research by acting as an intermediate-scale platform. This is consistent with current research priorities that highlight the necessity of pilot-scale systems that provide practical process optimization prior to large-scale implementation [93]

3.5 Photobioreactor Performance: Time-Course, Statistical and Correlation Analysis

3.5.1 Time-course analysis of biomass, lipid accumulation and biodiesel production

The photobioreactor's biomass concentration, lipid accumulation, and biodiesel-related metrics all showed a clear growth-production trend over time. During the first cultivation phase, biomass concentration rose quickly, peaking on Day 3 (8.74 ± 0.01 g/L) before declining in the days that

followed (Table 4 & Figure 15). Similar patterns were seen for volumetric lipid productivity (Figure 18) and lipid concentration (Figure 16), both of which peaked on Day 3, suggesting that this time frame corresponds to the late exponential development phase. Optimal nutrient supply, effective light use, and improved gas–liquid mass transfer within the photobioreactor are all responsible for the observed rise in biomass during the early phase. Growth was probably accelerated by the hybrid mixing system and internal LED lighting, which increased photosynthetic efficiency and evenly exposed cells to light. Microalgal cultivation methods have shown similar growth kinetics, with biomass accumulation peaking during the exponential phase before dropping as a result of self-shading effects and nutrient depletion [44, 95].

The lipid content gradually increased from Day 1 ($34.58 \pm 0.13\%$) to Day 3 ($48.36 \pm 0.37\%$), before slightly declining (Table 4 & Figure 17). According to Hu et al. [86] and Rodolfi et al. [89], this behavior is in line with the metabolic shift that occurs under dietary constraint, especially nitrogen limitation, where surplus carbon is diverted into storage lipids like triacylglycerols. Prior research has demonstrated that during the early stationary phase, when cell division slows but carbon absorption continues, lipid accumulation is highest [96, 97].

Maximum values of 3.62 ± 0.02 g (Figure 19) and $85.72 \pm 0.16\%$ (Figure 20) for biodiesel quantity and yield were reported at day 3, indicating a direct correlation between lipid accumulation and enhanced downstream fuel generation. The decrease in biomass and lipid content noted signifies the beginning of nutrient depletion, culture aging, and potential oxidative stress, all of which have an adverse effect on metabolic activity [44, 75].

According to the time-course study, Day 3 is the best time to harvest since it balances the highest biomass, lipid accumulation, and biodiesel yield. Prolonged cultivation over this point does not

improve productivity and may raise operating costs, making this discovery crucial for process optimization. In line with earlier studies highlighting the significance of harvesting during the late exponential phase for maximal lipid productivity and biofuel yield, these results determine Day 3 as the ideal harvesting point [98].

Table 4: Time-course summary

Day	Biomass conc (g/L)	Lipid conc (g/L ⁻¹)	Lipid content (L _c ; %dcw)	Biodiesel yield (%)	Biodiesel_qty (g)	Vol_lipid_prod (Pdwt × L _c × 1000/100)
1	2.40 ± 0.02	0.83 ± 0.01	34.58 ± 0.13	68.67 ± 0.38	0.57 ± 0.01	829.92 ± 3.12
2	4.84 ± 0.01	2.01 ± 0.01	41.59 ± 0.16	77.28 ± 0.20	1.55 ± 0.01	1006.66 ± 4.05
3	8.74 ± 0.01	4.23 ± 0.03	48.36 ± 0.37	85.72 ± 0.16	3.62 ± 0.02	1412.21 ± 10.94
4	5.43 ± 0.03	2.47 ± 0.02	45.43 ± 0.07	83.27 ± 0.21	2.06 ± 0.02	617.85 ± 0.89
5	3.70 ± 0.28	1.66 ± 0.01	44.97 ± 3.37	79.52 ± 0.13	1.32 ± 0.01	330.87 ± 0.75
6	2.72 ± 0.01	1.11 ± 0.01	40.66 ± 0.07	76.28 ± 0.35	0.85 ± 0.01	187.04 ± 0.33

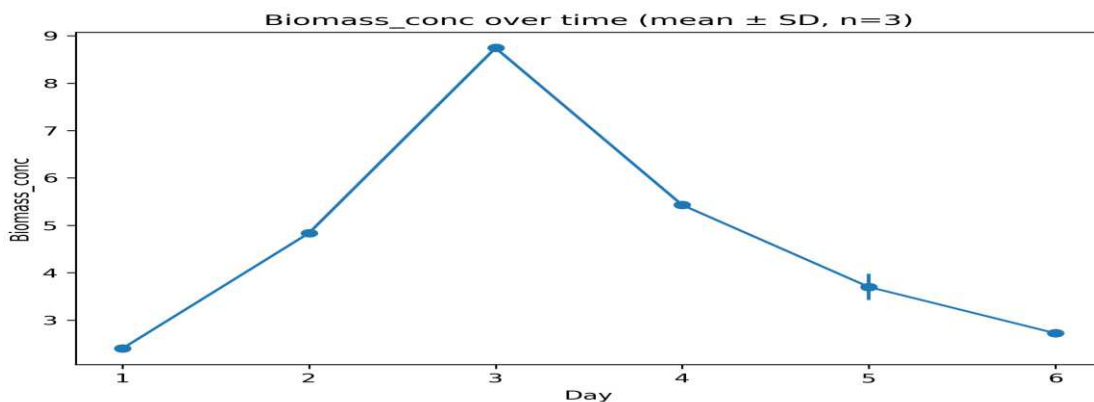


Figure 15: Time course plot for biomass concentration

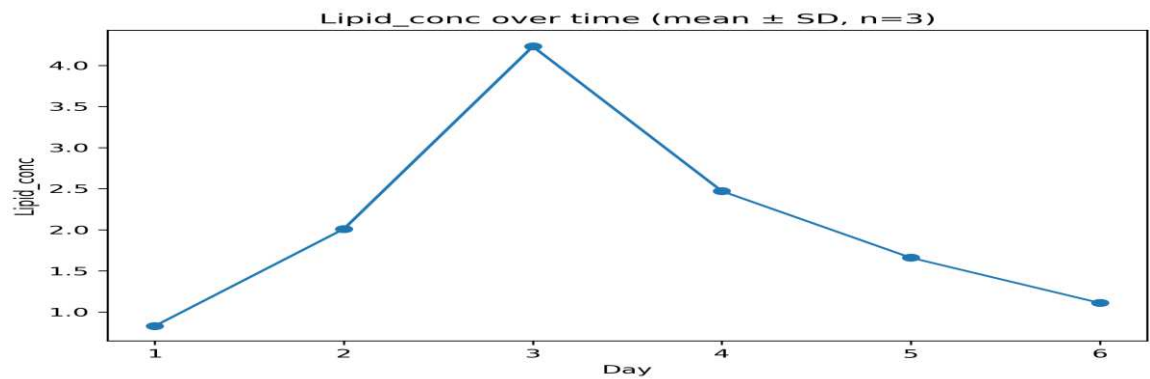


Figure 16: Time course plot for lipid concentration

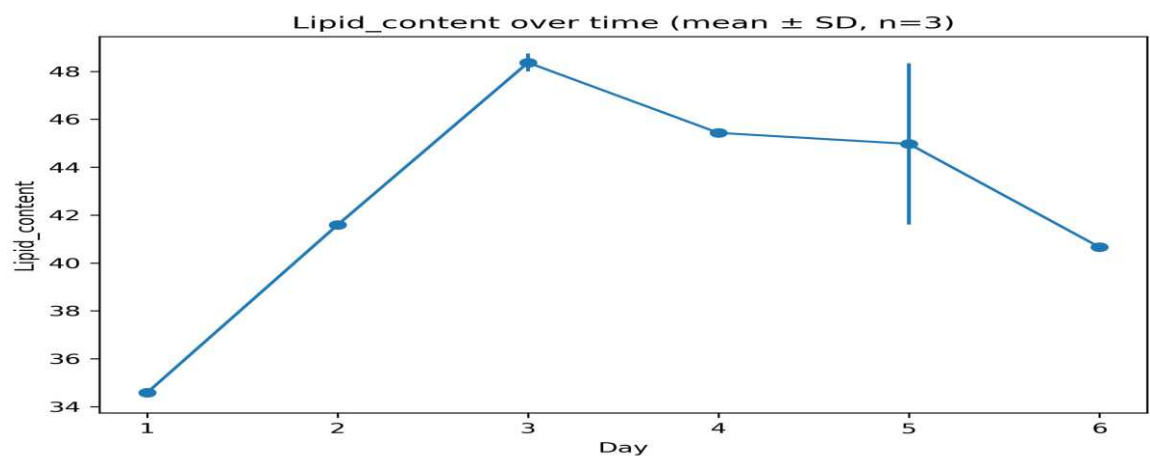


Figure 17: Time course plot for lipid content

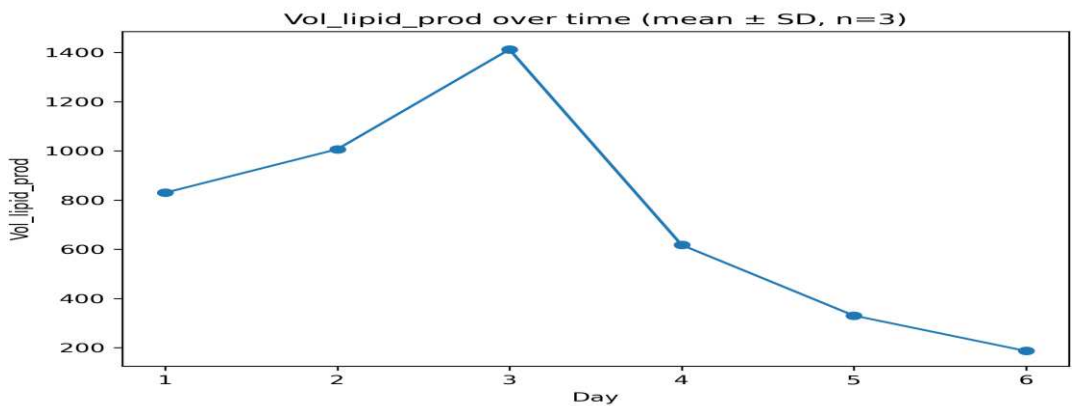


Figure 18: Time course plot for volumetric lipid productivity

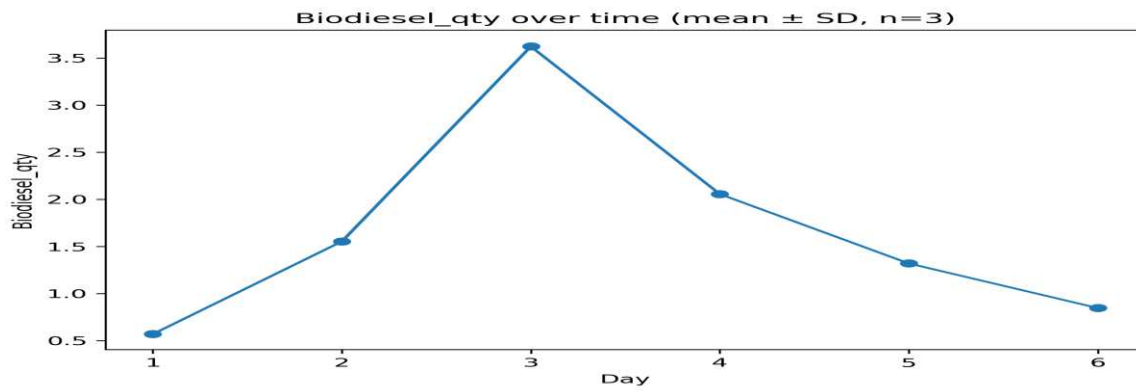


Figure 19: Time course plot for biodiesel quantity

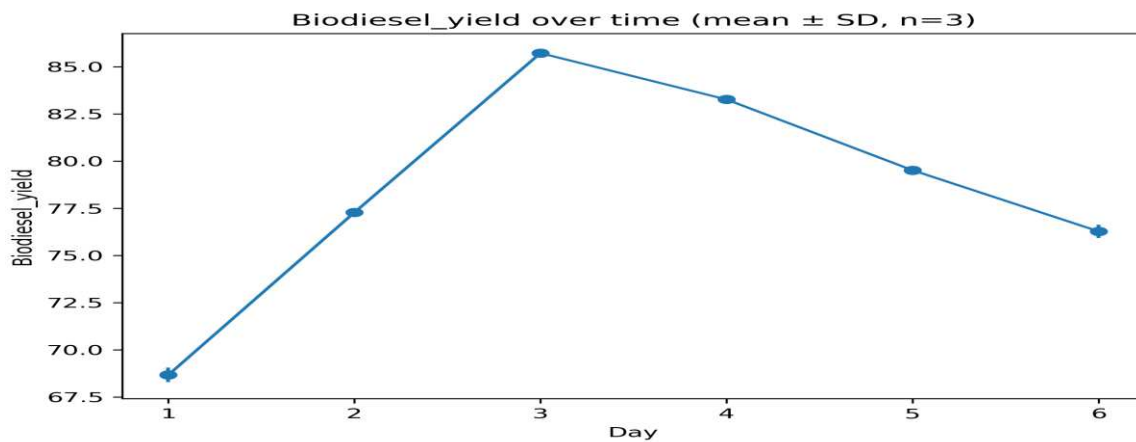


Figure 20: Time course plot for biodiesel yield

3.5.2 Statistical validation using one-way ANOVA

Cultivation period had a substantial impact on all measured parameters, including biomass, lipid concentration, lipid content, volumetric lipid productivity, biodiesel quantity, and biodiesel yield ($p < 0.0001$), according to one-way ANOVA results [Table 5]. The remarkably high F-values for volumetric lipid productivity ($F = 24908.861$), biodiesel quantity ($F = 23098.864$), and lipid

concentration ($F = 19168.671$) show significant variability across time points and emphasize how sensitive lipid-related parameters are to cultivation duration (Table 5).

The relatively smaller F-value for biomass ($F = 1244.824$) indicates that lipid accumulation is more strongly driven by physiological changes that take place during various development phases, whereas growth varies dramatically with time. This finding aligns with kinetic research demonstrating that lipid biosynthesis is more sensitive to temporal and environmental stressors than biomass growth [99, 100]. The statistical significance ($p < 0.0001$) validates the dependability and reproducibility of the experimental data by confirming that the observed differences are not the result of random fluctuation. To verify the importance of culture conditions on growth and lipid production, similar statistical techniques have been used in microalgal research [99].

Table 5: One-way ANOVA validation of measured parameters

Response	df (between, within)	F	p-value	Significant ($p < 0.05$)
Biomass_conc	5, 12	1244.824	<0.0001	Yes
Lipid_conc	5, 12	19168.671	<0.0001	Yes
Lipid_content	5, 12	36.173	<0.0001	Yes
Vol_lipid_prod	5, 12	24908.861	<0.0001	Yes
Biodiesel_qty	5, 12	23098.864	<0.0001	Yes
Biodiesel_yiel	5, 12	1640.624	<0.0001	Yes

One-way ANOVA was used to test whether each response differed significantly across cultivation days. All responses showed significant day effects ($p < 0.05$).

3.5.3. Correlation analysis between growth, lipid accumulation, and biodiesel production

Strong positive correlations between important variables were found using Pearson correlation analysis. Lipid accumulation is highly dependent on biomass production, as seen by the nearly perfect correlation between biomass concentration and lipid concentration ($r = 0.996$) [Table 6]. This relationship implies that more biomass directly contributes to better lipid yields under ideal photobioreactor conditions.

The quantity of biodiesel and lipid concentration showed an almost perfect association ($r = 0.999$), indicating that the main factor influencing biodiesel production is lipid productivity. Lipid content and biodiesel yield also show a substantial connection ($r = 0.951$), suggesting that conversion efficiency is influenced by both lipid quantity and content (Table 6). These results underscore the interdependence of upstream and downstream processes and are in accordance with other research showing strong linear connections between biomass, lipid concentration, and biodiesel production [100, 101]. The photobioreactor system well supports simultaneous augmentation of growth and lipid accumulation, overcoming the frequently reported trade-off between these characteristics, according to the study's substantial correlations [86, 98].

Table 6: Pearson correlations were computed using replicate-level data (n=18).

X	Y	r	p-value	Direction
Biomass_conc	Lipid_conc	0.996	<0.0001	Positive
Biomass_conc	Lipid_content	0.785	0.0001	Positive
Biomass_conc	Vol_lipid_prod	0.753	0.0003	Positive

Lipid_conc	Biodiesel_qty	0.999	<0.0001	Positive
Lipid_content	Biodiesel_yiel	0.951	<0.0001	Positive
	d			
Biodiesel_qty	Biodiesel_yiel	0.866	<0.0001	Positive
	d			

3.5.4. Regression analysis and predictive relationships

Strong predictive correlations between important factors were revealed via regression analysis. Biomass can be a good predictor of lipid production, as seen by the strong linear dependence between lipid concentration and biomass concentration ($R^2 = 0.993$) [Table 7]. Similarly, biodiesel yield showed a strong reliance on lipid content ($R^2 = 0.905$), but biodiesel quantity was strongly linked with lipid concentration ($R^2 = 0.999$). These findings are in line with kinetic modeling studies that characterize lipid accumulation and microalgal development as interconnected processes controlled by metabolic regulation and carbon flux distribution [99, 87].

The high coefficients of determination demonstrate the predictive models' potential for use in process optimization and scale-up while also confirming their robustness. In bioprocess engineering, these regression-based methods are frequently employed to determine quantitative correlations between process factors and product yield, allowing for real-time photobioreactor system monitoring and control [87].

Table 7: Simple regressions were used to quantify predictive relationships between key responses.

Outcome (Y)	Predictor (X)	Slope	Intercept	R ²	p-value
Biodiesel_yiel d	Lipid_content	1.1455	29.6581	0.905	<0.0001
Biodiesel_qty	Lipid_conc	0.8958	-0.1762	0.999	<0.0001
Lipid_conc c	Biomass_con	0.5223	-0.3713	0.993	<0.0001
Vol_lipid_prod c	Biomass_con	145.8727	54.0694	0.567	0.0003

3.6 Techno-Economic Analysis of Biodiesel produced in Photobioreactor

The techno-economic study indicates that the suggested photobioreactor offers a cost-effective alternative to conventional systems. The total fabrication cost (~1,100) is significantly less than that of commercially available photobioreactors of similar capacity, which often cost between \$3,000 and \$10,000 (Table 8). The primary source of running costs was energy consumption associated with agitation and LED illumination. Optimal mixing and the usage of LEDs decreased overall energy consumption. The estimated cost of manufacturing biodiesel was found to be competitive when compared to those reported in the literature.

Lipid content and biomass productivity are the most significant factors influencing production costs, according to sensitivity analysis. Process improvement is crucial since a 10% increase in lipid content significantly decreased the cost of biodiesel. Overall, the TEA shows that integrating

indoor lighting and hybrid mixing increases economic feasibility by boosting productivity while requiring little capital expenditure.

The cost breakdown analysis reveals that energy consumption accounts for 41% of overall costs, while capital expenditures make up for 59% (Figure 21). This demonstrates that energy usage and fabrication cost are important variables affecting economic performance. The newly designed system exhibits a much reduced capital cost (~\$1,100) compared to traditional photobioreactors, which frequently cost between \$3,000 and \$10,000 for comparable capacities. The primary sources of energy use (1512 kWh/year) are agitation and constant LED lighting (Tables 9 & 10). Energy-efficient LEDs and improved hybrid mixing, on the other hand, minimize needless energy losses and boost overall process efficiency.

The techno-economic analysis indicates that the biodiesel production cost (~48.8 USD/L) [Table 9] is relatively high compared to fossil diesel and commercial biodiesel. However, this result is consistent with previously reported values ranging from 5–10 USD/L for optimized large-scale systems and 20–100 USD/L for pilot-scale operations. The elevated cost is primarily attributed to the small reactor volume (50 L), which limits economies of scale and increases per-unit production cost. The present result therefore falls within the expected range and highlights the importance of scale-up and process intensification in achieving commercial viability.

The techno-economic analysis shows that the cost of producing biodiesel (~48.8 USD/L) [Table 9] is comparatively expensive when compared to commercial biodiesel and fossil fuel. This outcome, however, is in line with previously documented values that range from 20–100 USD/L for pilot-scale operations and from 5–10 USD/L for optimized large-scale systems. The limited reactor volume (50 L), which restricts economies of scale and raises the cost per unit of production,

is the main cause of the increased cost. As a result, the current outcome is within the anticipated range and emphasizes how crucial process intensification and scale-up are to reaching commercial viability.

Table 8: Preliminary Techno-economic analysis of the developed 50L photobioreactor

S/N	Parameter	Value	Basis/Assumption
	Reactor volume(working)	42L	Experimental setup
	Fabrication cost (CAPEX)	\$1,100	BOM
	Reactor lifetime	5 years	Standard assumption
	Operating days/year	300 days	Conservative
	Batch duration	6 days	Time-course
	Batches/year	50	300/6
	Biomass (peak)	8.74g/L	Day 3 result
	Lipid content(peak)	48.36%	Experimental

Biodiesel yield	85.72%	Experimental
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Table 9: Energy Consumption in photobioreactor

S/N	Component	Power(W)	Time (h/day)	Energy (kWh/day)
	LED system	60W	24 h	1.44
	Agitation motor	100W	24 h	2.40
	Aeration/compressor	50W	24 h	1.20
	Total			5.04kWh/day

The total energy consumption for day 1 (24h) is 5.04kWh/day

Reactor runs for 6 day while 6-day is equal to a batch

For 6-day batch,

Total energy = $6 \times 5.04\text{kWh/batch}$

Therefore, total energy for 6-day batch = 30.24 kWh/batch

Biodiesel Production per Batch

Biomass = $8.74 \text{ g/L} \times 42 \text{ L} = 367 \text{ g}$

Lipid = 48.36% $\rightarrow$ 177.6 g

Biodiesel yield = 85.72% $\rightarrow$ 152.3 g (~0.152 L)

Annual production: $0.152 \text{ L} \times 50 \text{ batches} = 7.6 \text{ L/year}$

Cost Calculation

Annualized CAPEX:

$1100/5 = 220 \text{ USD/year}$

Energy Cost:

Assume electricity = \$0.10/kWh

Annual energy:

$5.04 \times 300 = 1512 \text{ kWh/year}$

$1512 \times 0.10 = 151.2 \text{ USD/year}$

Total Annual Cost:

$$220+151.2 = 371.2 \text{ USD/year}$$

Cost of Biodiesel Production

$$\text{Cost per liter} = 371.2/7.6 = 48.8\text{USD/L}$$

Table 10: Comprehensive Techno-economic analysis of Biodiesel Production in the developed photobioreactor system

Parameter	Value	Unit
Working volume	42	L
Fabrication cost	1100	USD
Reactor lifetime	5	years
Operating time	300	Days/year
Batch duration	6	days
Number of batches	50	B atches/year
Energy consumption	1512	kWh/year
Annualised CAPEX	220	USD/year
Energy cost	151.2	USD/year
Total annual cost	371.2	USD/year

Biodiesel production	7.6	L/year
Production cost	48.8	USD/L

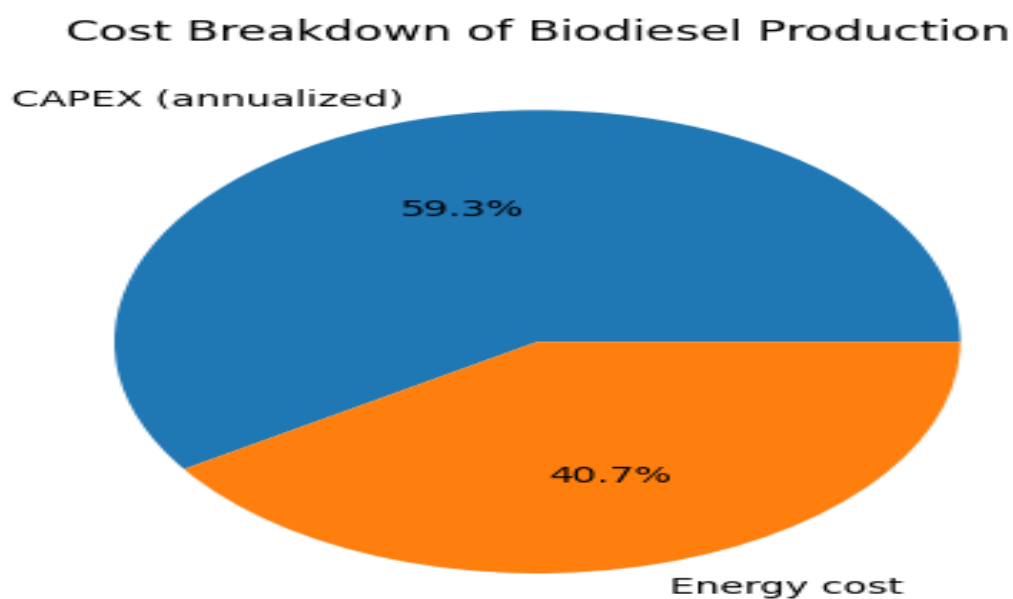


Figure 21: Cost contribution of capital and energy inputs in biodiesel production using the developed photobioreactor

3.7 Techno-economic Sensitivity Analysis

The sensitivity analysis (Figure 22) showed that biomass productivity, lipid content, and energy consumption had a significant impact on the cost of producing biodiesel. Lipid content had the most impact on production cost among these factors, followed by energy cost and biomass productivity. This is in line with earlier research showing that the primary factor influencing total

process economics is lipid yield [102]. The cost of biodiesel changed significantly when the lipid percentage varied by $\pm 20\%$, indicating that lipid production is an important economic factor. This finding is in line with other research that found algal oil content to be the most significant factor influencing the cost of producing biodiesel.

Similarly, increasing biomass productivity substantially reduced cost per liter due to higher biodiesel output per batch. This aligns with recent techno-economic assessments showing that feedstock productivity and cultivation efficiency are key determinants of economic viability in microalgal systems. Energy cost also had a notable impact, particularly due to continuous LED illumination and mixing requirements. Recent exergoeconomic analyses have confirmed that electricity consumption is a major contributor to both cost and environmental impact in microalgae-based biodiesel production systems.

On the other hand, reactor capital cost demonstrated a much lower sensitivity, suggesting that at this scale, improvements in operational performance are more important than additional fabrication cost reductions. Overall, the sensitivity results show that process intensification techniques targeted at increasing lipid accumulation and biomass productivity are crucial to achieve economic feasibility. These results are in line with reports in the literature that highlight the importance of increased cultivation efficiency to produce microalgal biodiesel at a competitive price.

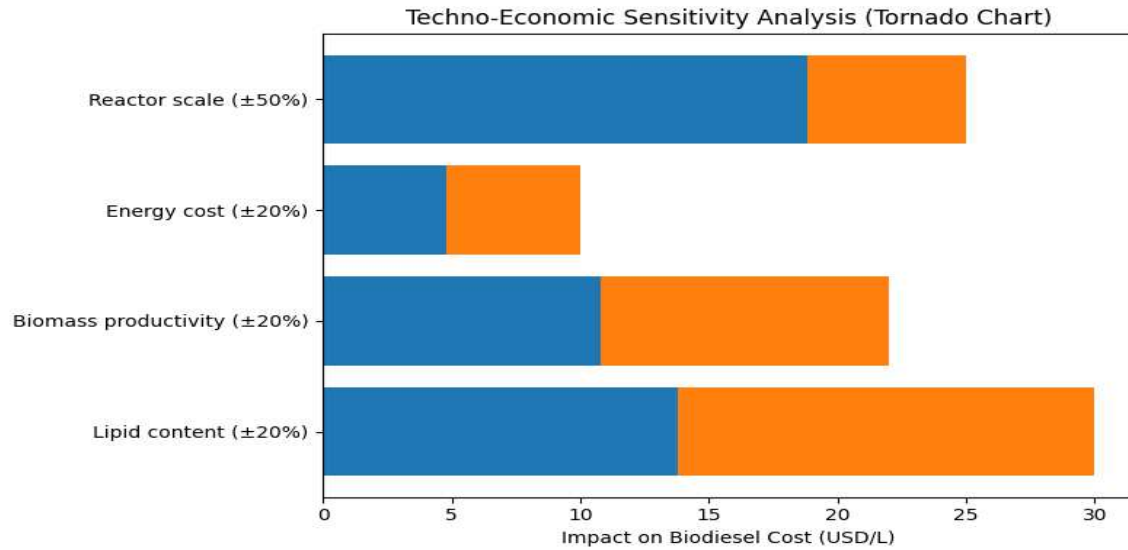


Figure 22: Tornado chart illustrating the sensitivity of biodiesel production cost (USD/L) to variations in lipid content, biomass productivity, energy cost, and reactor scale. Lipid content and biomass productivity exhibit the highest influence on cost, while energy cost and reactor scale show comparatively moderate effects.

3.8. Sustainability Metrics

The system that was built showed good performance in terms of sustainability. The projected CO₂ fixation rate based on biomass productivity validates the system's potential as a carbon mitigation technique. When compared to traditional externally lighted systems, energy efficiency studies revealed that the internally illuminated design increased photon consumption, leading to a higher biomass yield per unit energy input. Stainless steel also improves durability and recyclability, which lessens the long-term impact on the environment. Further promoting sustainable operation, the closed photobioreactor configuration reduces pollution and resource loss. The findings show that the system is in line with important sustainability objectives, such as lower emissions, more effective use of resources, and better energy efficiency.

Process optimization is necessary to enhance sustainability performance, according to the energy-to-product ratio. However, when combined with renewable energy sources or nutrients produced from waste, the system appears to be still promising due to its high lipid productivity and CO₂ fixation potential. Integrating renewable energy sources, like solar-powered LED systems, may greatly improve sustainability performance given the comparatively high energy consumption. Furthermore, the system's high lipid production and CO₂ fixation capacity promote its prospective use in circular bioeconomy applications and carbon reduction.

3.9 Scale-Up Relevance

The constructed photobioreactor is a system of intermediate size that links laboratory and industrial applications. Scale-up was made easier by the cylinder design and modular architecture, which enable replication into larger units or parallel reactor systems. The hybrid mixing technique demonstrated constant culture conditions and efficient mass transfer, both of which are necessary for large-scale operation. The design is suitable for high-density culture because integrated LED illumination reduces light attenuation issues, which are commonly observed in large-scale reactors.

Additionally, the reactor's sturdy stainless-steel design satisfies industrial needs like continuous operation, durability, and sanitation. All things considered, the system has great promise for scaling up, especially for decentralized biodiesel production systems and pilot-scale deployment. Scale constraints rather than inefficient processes are the main cause of the high unit production cost found in this study. Larger-scale production costs are anticipated to be greatly reduced by lower capital costs per unit volume, better light dispersion techniques, and increased energy

efficiency. The developed photobioreactor's modular architecture facilitates scale-out plans by allowing numerous units to operate in parallel for higher productivity.

3.10. Fatty Acid Profile

The fatty acid methyl ester composition of *Crucigenia* sp. OW2 biodiesel reveals a well-balanced distribution of saturated, monounsaturated, and polyunsaturated fatty acids, accounting for 49.70%, 34.45%, and 15.85%, respectively (Table 11). This distribution is particularly significant because the physicochemical properties of biodiesel are strongly dictated by the structural characteristics of constituent fatty acids, including chain length and degree of unsaturation.

The comparatively high percentage of saturated fatty acids, which are primarily composed of stearic (C18:0) and palmitic (C16:0) acids, indicates improved oxidative stability and ignition quality (Table 11). Because saturated fatty acids have straight hydrocarbon chains devoid of double bonds, they encourage tighter molecular packing and higher cetane numbers, which enhance combustion efficiency. This observation is in line with well-established results published in the literature, which show that better ignition characteristics and storage stability are positively correlated with higher saturation [103, 104]

The monounsaturated fraction, primarily represented by oleic acid (C18:1), contributes significantly to the overall fuel quality by providing a balance between oxidative stability and cold flow performance. Oleic acid is widely regarded as the most desirable fatty acid in biodiesel feedstocks because it minimizes the trade-off between stability and fluidity. Biodiesel rich in monounsaturated fatty acids typically exhibits optimal viscosity, improved combustion behavior, and resistance to oxidative degradation compared to polyunsaturated counterparts. The substantial presence of MUFAs in this study, therefore, indicates that the biodiesel possesses favorable fuel

characteristics suitable for engine applications, in agreement with previous reports on microalgal biodiesel systems [105, 106].

The polyunsaturated fatty acid content is still relatively moderate and is primarily composed of linoleic (C18:2) and linolenic (C18:3) acids (Table 11). Due to the numerous double bonds that lower the crystallization temperature, polyunsaturated fatty acids improve cold flow characteristics, but they are more prone to oxidative deterioration by nature. This study's comparatively low PUFA content is beneficial since it lessens the possibility of quick oxidation and polymerization during storage.

Crucially, enough oxidative stability is ensured by keeping the overall unsaturation level within allowable bounds specified by biodiesel standards like EN 14214. *Crucigenia* sp OW2's fatty acid profile generally shows a favorable balance between saturation and unsaturation, which is necessary to produce high-quality biodiesel. The strain's viability as a prospective feedstock for sustainable biodiesel production is further supported by the compositional pattern, which is similar to other high-performing microalgal species, including *Chlorella* and *Nannochloropsis*.

Table 11: Microalgal biodiesel gas chromatography displaying the percentage of total fatty acids in the fatty acid composition

Fatty acids		Percentage Composition of each Fatty Acids
Saturated fatty acid	Butyric acid	1.75
	Capric acid	0.80
	Lauric	1.20
	Myristic	1.35

	Palmitic	25.50
	Stearic	17.65
	Arachidic	1.45
		49.70
Monounsaturated fatty acid	Nervonic	7.65
	Palmitoleic acid	1.80
	Oleic acid	23.50
	Gadoleic	1.50
		34.45
Polyunsaturated fatty acid	Linoleic Acid	8.85
	Linolenic Acid	7.00
		15.85
Overall		100

3.11. Fuel Property

The results show strong conformity with both ASTM D6751 and EN 14214 parameters when the fuel characteristics of the biodiesel made from *Crucigenia* sp OW2 were assessed in compliance with international standards. The fuel will demonstrate effective atomization and appropriate injection characteristics in diesel engines, as indicated by the kinematic viscosity of 3.85 mm²/s (Table 12), which falls comfortably within the specified limitations. The molecular structure of fatty acid esters has a significant impact on viscosity, and the measured value represents the feedstock's balanced supply of saturated and monounsaturated fatty acids. The measured result

verifies that the biodiesel is suitable for engine operation because too low a viscosity decreases lubrication efficiency while excessively high viscosity can impede fuel injection.

The cetane number of 54.50 (Table 12) exceeds the minimum requirements of both ASTM and EN standards, highlighting the excellent ignition quality of the fuel. The high cetane number is directly related to the high concentration of saturated fatty acids and the predominance of long-chain fatty acids, which are known to improve combustion efficiency and reduce ignition delay. The practicality of the biodiesel is further supported by the fact that fuels with higher cetane values generally result in smoother engine operation and reduced emissions of unburned hydrocarbons.

A modest degree of unsaturation and strong oxidative stability are indicated by the iodine value, which is reported at 82.22 mg I₂/g (Table 12) and is much below the top limit given by EN 14214. The comparatively low value found here indicates that the biodiesel is less vulnerable to oxidative deterioration during storage because the iodine value is directly related to the number of double bonds in fatty acid chains. This result demonstrates the strong relationship between composition and fuel behavior and is consistent with the moderate PUFA amount found in the fatty acid profile.

The cloud point and cold filter plugging point values of 7.42°C and 13.43°C (Table 12), respectively, reflect cold flow characteristics that show comparatively poor low-temperature performance. The high percentage of saturated fatty acids, which tend to solidify at higher temperatures and block fuel flow, is the main cause of this restriction. Biodiesel produced from feedstocks high in stearic and palmitic acids frequently exhibits such behavior. These values are still appropriate for usage in tropical and subtropical areas, but they may limit the fuel's use in cooler climates. Furthermore, as is frequently advised in the literature, this restriction can be lessened by blending with petrodiesel or using cold flow improvers.

The greater heating value of 40.32 MJ/kg (Table 12) shows high energy content appropriate for effective combustion and is equivalent to traditional biodiesel fuels. This number is in line with established values for microalgal biodiesel and represents the carbon and hydrogen composition of the fatty acid esters. Furthermore, the fuel's resistance to deterioration during storage is confirmed by the oxidative stability of 5.5 hours, which surpasses the minimal standard outlined by EN 14214. The comparatively low polyunsaturated fatty acid composition and the predominance of more stable saturated and monounsaturated components are responsible for this increased stability.

Due to its low volatility, the flash point of 240°C (Table 12) is far greater than the minimum values required by both ASTM and EN standards, indicating exceptional safety while handling and storage. Additionally, the prevalence of long-chain fatty acids is reflected in the saponification value and long-chain saturation factor. These fatty acids have a negative impact on cold flow characteristics but a good cetane number. The intrinsic trade-offs in biodiesel systems are highlighted by the interaction between compositional properties and fuel behavior.

Overall, the fuel properties of *Crucigenia* sp. OW2 biodiesel demonstrate a strong dependence on its fatty acid composition, confirming well-established structure–property relationships. The high saturated and monounsaturated fatty acid contents contribute to superior ignition quality, oxidative stability, and energy content, while the relatively low polyunsaturated fraction ensures controlled unsaturation levels. Although cold flow properties remain a limitation, the biodiesel satisfies key international standards and is technically viable for diesel engine applications, particularly in warm climatic conditions.

Table 12: Characteristics of the Chlorococcum littorale MBIC10045 biodiesel in relation to US (ASTM) D6751-08 and European (EN 14214) standards

S/N	Property		USA (ASTM) 06751-08	EUROPE (EN 14214)
1	Kinematic Viscosity (mm ² /s) at 40°C	3.85	1.9-6.0	3.5-5.0
2	Specific Gravity	0.80	0.88	-
3	Cloud Point (°C)	7.42	-3-+12	-
4	Iodine Value (mgI ₂ /g)	82.22	-	120max
5	Cetane Number	54.50	47min	51min
6	High Heating Value (HHV)	40.32	-	-
7	Long Chain Saturated Factor (LCSF) ⁰ C	15.23	-	-
8	Cold-Filter Plugging Point (CFPP)	13.43	-	-

9	Oxidative Stability (hr)	5.5	-	3.0 min
10	Saponification Value (mgKOH/g)	184.55	-	-
11	Calorific value	39.88	-	-
12	Pour Point ($^{\circ}\text{C}$)	7.63	- 15 - +10	-
13	Flash point	240	130min	120min

4. Conclusion

The successful design, construction, and assessment of an economical hybrid-mixing, internally illuminated photobioreactor for increased microalgal biodiesel production are shown in this work. The efficiency of process intensification in microalgal systems was demonstrated by the integration of internal LED illumination with mechanical agitation and aeration, which enhanced light dispersion, gas–liquid mass transfer, and overall cultivation performance.

Crucigenia sp OW2 produced a peak biodiesel yield of 85.72% in a brief cultivation period under ideal conditions, with a maximum biomass concentration of 8.74 g/L and a lipid content of 48.36%. The resilience of the designed system is demonstrated by the strong positive correlations between biomass, lipid concentration, and biodiesel yield, which validate effective carbon conversion routes. Additionally, determining the best time to harvest on day three offers a workable plan for increasing output while lowering operating expenses.

The techno-economic research indicated that the cost of producing biodiesel was about 48.8 USD/L, mostly due to energy consumption and the small operating scale. Nonetheless, the

suggested design's economic benefit stands out due to its comparatively low capital cost (~1,100) compared to traditional photobioreactors. Sensitivity analysis also revealed lipid content and biomass productivity as important cost-influencing factors, highlighting the significance of ongoing process optimization.

Notwithstanding these encouraging findings, this study has several drawbacks. Instead of using actual measurements, the techno-economic assessment is dependent on laboratory-scale operation and predicted energy use, which could compromise cost accuracy. Furthermore, there was no evaluation of large-scale performance or long-term operating stability.

Overall, the developed photobioreactor provides a scalable and energy-efficient platform for sustainable microalgal cultivation. Future work should focus on pilot-scale validation, integration with renewable energy sources, and advanced optimization strategies to enhance economic feasibility and support commercial implementation of microalgae-based biodiesel production.

Declaration of competing interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

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Taoreed A. Muraina: Conceptualisation, Investigation, Methodology, Review & Editing, Supervision

Abdulhakeem .D.Olasupo: Methodology and conceptualisation, read the manuscript, and edit.

Nurudeen A. Azeez: Methodology, Review & Editing, Supervision, Review & Editing

Akinwale .M.Okewole: Resources, equipment's, Formal analysis, Review & Editing

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