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Investigation of the effect of lake sediment and solar-integrated photo-bioreactors on the lipid production from spirulina platensis

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ABSTRACT

The main objectives of the current research assess how engineering and environmental factors affect the production of lipids oil from Spirulina (*Arthrospira platensis*) in solar-integrated photo-bioreactors. In addition, the effects of media type (freshwater vs. seawater), sediment concentration 10 g/l/day (1%), and cultivation duration (4–18 days) on microalgal growth and lipid accumulation were evaluated using laboratory-scale (1 L and 6 L) and outdoor modular (6 × 18.9 L) photo-bioreactors. Sediment concentration decreased lipid percentage by about 10% for every 10 g/l/day more sediment (1%), whereas freshwater media consistently produced higher lipid content, reaching a maximum of 7.96±0.04% after 18 days. The results indicate that integrating solar-powered photo-bioreactors for sustainable microalgae lipid production is technically feasible, underscoring the significance of reactor design and environmental optimization for biofuel applications. In laboratory settings, LED lighting greatly improved lipid synthesis, and freshwater media consistently performed better than seawater. Model 1 showed very high predictive accuracy ($r \geq 0.999$) for freshwater systems in both the lab and the field. Freshwater yields steadily increased over time, whereas seawater production decreased significantly after 14 days. These results indicate that using freshwater media and Model 1 together is the best way to obtain the most lipids. The study presents controlled sediment dosing as a new strategy for microalgal cultivation with potential for low-cost and sustainable nutrient supply, such as the traditional approaches based on synthetic media or wastewater supplementation and environment-friendly biofuel production systems. Furthermore, this study investigated the direct application of natural sediments to stimulate lipid accumulation.

Keywords: lipid, photo-bioreactors, sediment supplementation, algal, spirulina, freshwater, seawater, protein.

INTRODUCTION

External pollution in lakes is strictly controlled; climate change is more and more often causing eutrophication phenomena in water bodies and record- setting algal blooms, according to a recent study from the European Commission. These occurrences highpoint the huge biological productivity of cyanobacteria under fluctuating climatic conditions, while also flagging an increasing ecological threat. This inherent productivity can be taken advantage of by switching from these uncontrolled environmental algal blooms to the controlled cultivation of Spirulina platensis in solar-integrated photo-bioreactors. This strategy makes straight ecological urgency with innovative bioengineering solutions by converting a key environmental challenge into a sustainable, ecofriendly, and carbon neutral source of high value lipids (Istvánovics et al., 2022; Science for Environment Policy, 2023). Along with the consequences of nutrient variations brought

on by climate change, the interaction of chemical and physical stressors complicates algal physiology. According to previous research, pollutants, for example, polycyclic aromatic hydrocarbons (PAHs), are the main inhibitors of algal concentration and photosynthetic effectiveness. Additionally, by raising oxidative stress and lowering nutrient assimilation, physical turbulence and sediment resuspension can worsen these effects. Conversely, the design of more successful engineering interventions is made possible by an understanding of these physical, chemical, and biological relationships, particularly how stressors affect elemental stoichiometry and photosystem II (PSII) performance. To minimize these inhibitions and optimize the conditions required to take full advantage of lipid production from Spirulina platensis as a feasible and ecofriendly solution for the future bio-economy, solar-integrated photobioreactors can be used to create a controlled environment (Lo et al., 2025).

Microalgae provide high yields of lipid without competing with agriculture, making them an encouraging feedstock for next-generation biofuels (Chisti, 2007; Scott et al., 2010). Commercialization is hampered by the problems with lipid extraction and productive growth, despite improvements in cultivation systems (Molina et al., 2003). Microalgae-derived biofuels, which have benefits for the environment and energy security, are becoming more popular as the world moves toward sustainable energy sources (Sehsah et al., 2015). Microalgae do not compete for arable land, in contrast to the first generation of biofuels that depend on food crops (Pulz, 2001). However, the high energy and operational costs of cultivation, harvesting, and lipid extraction present challenges for large-scale commercialization. Although photo-bioreactors (PBRs) provide controlled growth, their energy requirements may overshadow any environmental benefits.

Microalgae cultivation for bioenergy requires optimization of PBRs engineering, nutrient, and environmental parameters. Integrated microbial fuel cell-photobioreactor (MFC-PBR) configurations have been shown to significantly improve bioenergy production and carbon sequestration, by linking the degradation of exoelectrogenic bacteria with the photosynthetic growth of microalgae (Salim et al., 2025). Metabolic pathways are not only shaped by system mechanics, but also by cultivation stressors. Salinity level adjustments within a batch PBR are, for example, an important osmotic trigger altering growth kinetics and promoting cellular accumulation of lipids for biodiesel feedstock (Khadim et al., 2024). In addition, progressive adaptation strategies that enable robust strains to tolerate and grow effectively on harsh industrial waste matrices, such as oilfield-produced water, have been developed. These studies show that a controlled environment of PBRs can be utilized to combine high-yield lipid production with active wastewater remediation (Ammar et al., 2018).

Biofuel represents a potential environmental and energy security benefit. However, it poses sustainability concerns, as it depends on food crops as feedstocks (Sehsah et al., 2015). By integrating solar photovoltaics (PV) into PBRs systems, greenhouse gas emissions and operating expenses can be decreased, encouraging the production of sustainable biofuels (Carvalho et al., 2006). For example, Egypt's Lake Burullus needs extensive dredging to remove sediments (sand, silt, and clay) that is not currently useful (Hany et al., 2022). In addition, reducing energy inputs by using solar energy to power microalgae cultivation could help with these issues (Pulz, 2001). By integrating PBRs with solar PV panels, hybrid systems can reduce dependence on conventional (fossil fuels) energy by providing electricity for mixing, lighting, and temperature control (Singh and Gu, 2010). To make the most out of resource recovery, residual streams from growing microalgae should go through thorough environmental impact assessments to see if they can be turned into agricultural fertilizers, livestock feed, or substrates for making biogas (Green et al., 2024).

There are differences between freshwater and saltwater usage, and water needs are crucial. Closed-loop systems and treated wastewater are two examples of water recycling and reuse that can drastically lower the total environmental damage of water and supply nutrients for algal growth (Johnson et al., 2022). In saline environments, lipid buildup may require customized management techniques (Converti et al., 2009). Moreover, natural sediment can be a source of nutrients (Ugwu et al., 2008). Seawater is plentiful for large-scale outdoor systems, although high salinity can impact lipid accumulation; freshwater promotes faster growth (Chinnasamy et al., 2009). Vertical farming or cultivation on marginal land (Lee et al., 2021) can reduce Land-use impact. This study used waste sediment from underwater sediments excavation operations to investigate

the production of lipid-based biofuel from A. platensis using solar-integrated photobioreactors.

According to the Abou-Shanab et al. (2013), sediment can support microalgal cultivation and solar integration will result in higher lipid yields because of lower energy costs. In order to test these theories over an 18-day period, utilizing freshwater and seawater with additional sediment stress, a modular laboratory and outdoor PBR system was created. Growth, protein, and lipid percentages were measured to help design large-scale and energy-efficient photo-bioreactors.

Optimizing algae cultivation for lipid production requires a two-stage approach that separates fast biomass growth from high lipid accumulation, commonly achieved through nutrient starvation or stress induction. Specific "sediment dosing" as a source of nutrients is less common in the mainstream literature but saline wastewater, industrial effluent and iron supplementation are used as innovative, low-cost, "dosing" strategies to improve both biomass sedimentation and lipid accumulation. Under stress conditions like high salt, high light, or nutrient limitation, microalgae can overproduce lipids or carotenoids (Gong and Bassi, 2016; Singh et al., 2016). For example, lipid accumulation in *Dunaliella* sp. and *Chlorella vulgaris* was significantly enhanced under high-salinity stress, reaching 70% and 21.1%, respectively (Duan, 2012 and Takagi et al., 2006). Similarly, the β-carotene yield of Dunaliella salina was increased 30-fold when salt concentration was increased from 4 to 9% (Coesel et al 2008). However, the stress-based strategies generally have a negative impact on cell growth and also reduce the yield of desired products. The existing literature was mainly focused on the effect of various stresses on the production of lipids or carotenoids or has discussed the benefits of metabolic engineering for the improvement of microalgae strain (Jagadevan et al., 2018 and Banerjee et al.,2016) To reduce the production cost and improve the lipid productivity, microalgae should be cultivated simultaneously in low-cost cultivation systems, such as co-cultivations (Kouzuma and Watanabe, 2015). Microorganisms are capable of rapid adaptation to changing environments.

Under high stress conditions, adaptation can be achieved through the acquisition of beneficial phenotypes via random genomic mutations and their positive selection (Kim et al, 2014). Thus, it is not unexpected that the plasticity of microorganisms has been harnessed for the improvement of growth under stress conditions (Lu et al., 2012). Of the environmental stresses, the most studied was the adaptation of microalgae to unfavorable light conditions. The economic viability of microalgae is significantly constrained by biomass productivity of microalgae, especially in photosynthesis. In the ALE (adaptive laboratory evolution) process of 114 days, *C. vulgaris* was cultivated using cheaper LEDs at 660 nm compared to the traditional but more expensive LEDs at 680 nm (Fu et al., 2012). In most of the microalgae, high salt stress promoted lipid accumulation, but it usually caused the decrease of photosynthetic pigments and oxidative damage. To obtain salt stress-tolerant microalgae, ALE was conducted for 1255 generations towards high salt stress (200 mM NaCl) in the green alga *C. reinhardtii*, and the obtained strains could grow in high salt medium as fast as the progenitor strain under normal conditions. The evolved cells were able to accumulate similar amounts of lipid as the progenitor strain when nitrogen was depleted (Perrineau et al., 2014). This was accompanied by significant up-regulation of calmodulin protein and down-regulation of glycerol/phospholipid acyltransferase and transporters. This phenomenon was also in agreement with a previous study (Siaut et al., 2011).

Recently, salt-resistant strains of *Chlamydomonas* sp. were bred by a combined ALE and mutation strategy. It was found that the biomass production under high salinity was dramatically improved in the salt-resistant strains, but the lipid accumulation was decreased (Kato et al., 2017). However, the evolved strain achieved a maximum biomass of 134.5 g/L and lipid yield of 80.14 g/L when using the ALE strategy combined with 30 g/L NaCl in marine microalgae *Schizochytrium* sp., corresponding to a 32.7% and 53.31% increase compared to the parental strain, respectively (Sun, et al., 2018). The main limitation of the ALE strategy is that most of the results are microalgae specific and its outcome may be different from strain to strain, despite several benefits.

The net greenhouse gas (GHG) emissions associated with algae biofuel production should be measured in the carbon footprint analysis. This requires a thorough evaluation considering the emissions from every stage, including dewatering, harvesting, lipid transesterification, extraction, transportation, and cultivation. In addition, energy for mixing, nutrient production, and CO_2 supply.

An energy-equivalent comparison with traditional fossil fuels is essential. This comparison should reveal the possibility of considerable decreases in greenhouse gas emissions, particularly when considering algae's capacity to sequester CO_2 through the photosynthesis process. For instance, using CO_2 from industry in algae cultivation can further lower the net carbon intensity of biofuel.

The analysis should consider both direct emissions (like CO_2 from processing and N_2O from fertilizer use) and indirect emissions (like embodied energy in chemicals and infrastructure). The goal is to demonstrate how algae biofuels can help mitigate climate change by replacing fuels derived from fossil fuels (Smith et al., 2023).

Algae-based fuels are classified as a third-generation energy source, which has the potential to reduce the harmful effect of fossil fuels. The first and second generations have disadvantages, such as greenhouse gas emission and food safety (Brennan and Owende, 2010). There are two methods to cultivate algae: open pond and photobioreactor (Sheehan et. al, 2003). Open ponds are cheaper than photobioreactors, but contamination cannot be stopped. A photobioreactor does not need more land than open ponds, but both methods need nutrients and CO_2 as the main inputs for growing. A photobioreactor is a better way to boost the productivity of algae biomass (Sheehan et al., 2003). Algae, on the other hand, are very good at photosynthesis and can grow in wastewater (Mandal and Mallick, 2009). It takes a lot of water to produce one gallon of algae biofuel, about 5500 to 9100 gallons (Assmann et al., 2011). Moreover, this water cannot be recycled. On the other hand, biorefinery systems can keep the pH level of water at 7.0 (Yang et al., 2011). Six-unit photobioreactors use 183 tons of CO_2 every year to make 136 m^3 of oil per hectare. This finding demonstrates the beneficial effects of algae cultivation on the environment (Chisti, 2007). Recent laboratory analyses indicate that anaerobic digestion (AD) is the most suitable system for modifying photobioreactors. The AD unit does not require a harvesting process and can utilize deeper levels of algae biomass, because it can control itself and automatically provide the nutrients it needs (Bohutskyi and Bouwer, 2012).

Biofuel production from algae biomass is technically feasible and cost competitive (Medipally et al., 2015]. A higher profit can be obtained if a number of challenges are addressed to deal with the high expectations from algal biotechnology. Chisti (2007) indicated that, in contrast, algae have low lipid content; this characteristic makes harvesting process difficult. Therefore, the production costs high and the final product may not compensate for the expenses. However, the expenses could be reduced in half if production increased to 10.000 ton per year. The primary aim of the present study was to investigate the feasibility of generating lipid-based biofuels from A. platensis, while integrating photovoltaic solar energy into the processing system to function as a sustainable energy source for the conversion of biomass, particularly A. platensis, into biofuels. This study assessed the growth of *A. platensis* utilizing sediment, intending to exploit this waste material from dredging operations while evaluating its capacity to facilitate microalgal cultivation for biofuel production. The results could show that it is possible to combine sediment management with the systems that use renewable energy. The authors built a modular photobioreactor system for both indoor and outdoor use to test these ideas over 18 days. It used freshwater and seawater media with added sediment stress. Growth and protein content as well as lipid percentages were quantified. This work aimed to bridge knowledge gaps around maximizing micro-algal biofuel productivity through integration with solar energy in farming systems. Findings could help design energy-efficient, large-scale photobioreactor configurations (Carvalho et al., 2006; Chisti, 2007). This study was unable to evaluate the productivity rate of algae for the specified available area. In large-scale tests, though, it is possible to achieve high productivity with low operating costs.

MATERIALS AND METHODS

Experimental design

Two experimental designs were utilized. One took place in a lab under a controlled environment. The second one was a field-scale experiment done outside in a semi-controlled setting. Figure 1 shows that each phase was made to see how adding sediment, using solar-powered systems, and changing the type of water (freshwater versus saltwater) affected algal growth and lipid accumulation.

Laboratory experimental setup

The experimental system was housed in a rectangular box that was 30 × 25 × 50 cm. There

Figure 1. The photobioreactor set up for assessing outdoor algal biofuel production

were two main culture tanks in the setup, each with a 6 liter capacity. One had freshwater and the other had seawater. *Arthrospira* (Spirulina) platensis was added to both tanks at a starting concentration of 1g/l. To help algae grow, sediment that had been air-dried and collected from Lake Burullus, Egypt, was added every day at a rate of 10 g/l/day or 1% concentration.

During the 20-day growing period, the internal temperature stayed the same and the plants kept growing under a 24-hour LED light cycle. The system was powered from a built-in renewable energy unit that included a solar panel that uses photovoltaic (PV) technology, an inverter for power, and a set of electrical switches

Two more 6 L tanks were used as controls to set a baseline for comparison: Control 1 (freshwater) and Control 2 (seawater). These control units were kept under the same conditions as the experimental cultures, but they were not inoculated (no algae were added).

Field experiment setup

At the experimental farm, a photo-bioreactor system was set up to assess outdoor algal biofuel production. Eight cylindrical containers (18.9 L each), each holding 114 liters, three filled with freshwater with capacity 57 liters and three with seawater as well as two cylindrical containers used as experiment control with the same capacity. Control 1 was filled with fresh water, and control 2 was filled with seawater. Then, ¾ inch plastic hoses with couplings were used to connect the parallel containers, which were spaced 20 cm apart. For aeration to remove the CO_2, each container had holes connected to air hoses. Spirulina platensis algae was cultivated in each tank by 1 g/l concentration. The algal growth was supported by the addition of 10 g/l/day of air-dried Burullus sediments. Moreover, the control units were kept under the same conditions as the experimental cultures, but no algae were added.

The system was maintained in a transparent greenhouse (125 × 65 × 80 cm) that was supported by metal frames and served as a passive solar collector to control the temperature. A Calpeda VICENZA water pump (0.5 hp) was used to circulate and mix the water. A Davis Vantage Pro2 weather station was set up at the Faculty of Agriculture to continuously record meteorological data, such as temperature and relative humidity. A KS-235P photovoltaic panel with DC output converted to AC via a 1500-Watt DAKTM inverter, which provided power for the system.

This supplied energy to the control units, LED lighting, and heating components. Illumination within the photo-bioreactors was measured using an ST2232 Environmental Light Meter, solar irradiance with a TES-1333 Solar Power Meter, and daily electricity consumption with a digital wattmeter, based on lighting and heating requirements in both lab and field test trials.

Spirulina cultivation

Both laboratory setups were used to cultivate spirulina to assess its potential for producing lipids under stress caused by temperature fluctuations and sediment supplementation, as well as to mimic nutrient fluctuation, which is known to stimulate lipid accumulation in microalgae, sediment was added every two days. The sediment samples were examined for total phosphorus (TP), inorganic phosphorus (IP), organic phosphorus (OP), and total organic carbon (TOC)

prior to supplementation. As well as, to evaluate the system's operational efficiency and scalable potential, solar-powered biofuel production, power and energy consumption were simultaneously observed. Additionally, the algal samples were collected from saltwater and freshwater tanks every two days for biochemical analysis with an emphasis on lipid content.

Sediment preparation and analysis

For evaluating the impact of sediment on algal growth and lipid production during a two-week cultivation period, sediment was added by 10g/l/day (1%) concentration to each container. Furthermore, a modified dry ashing method outlined by Aspila et al. (1976) was implemented to measure organic phosphorus (OP) and inorganic phosphorus (IP) as well as total phosphorus (TP). The sample was burned at 550 °C and then extracted using 1 N HCl. OP was computed as the difference between TP and IP, and IP was measured directly from acid extracts. The ascorbic acid-molybdenum blue colorimetric method was used to measure phosphorus concentration calorimetrically (Murphy and Riley, 1962). The Loss on Ignition (LOI) method, conducted at 550 °C for four hours, was used to determine total carbon content in the collected sediment sample (Heiri et al., 2001). The analysis of sediment samples content in Laboratory of National Institute of Oceanography and Fisheries, NIOF gave the following values: TP (757 µg/g), IP (694 µg/g), OP (63 µg/g), and TOM (4.28%).

Algal chemical analysis

The algal biomass samples were examined for protein, lipid, moisture, ash, and carbohydrate content using the standard methods (AOAC, 1995). To measure moisture content, samples were dried at 105 °C to a constant weight. Total nitrogen was measured using the Kjeldahl method where the algal samples were digested in a Micro Kjeldahl apparatus, subjected to condensation, and titration. Finally, the total nitrogen content was multiplied by factor (6.25) to estimate protein. Lipids were extracted for eight hours in a Soxhlet apparatus using a 2:1 chloroform methanol solvent mixture. The dried biomass was burned for six hours at 550 °C in a muffle furnace to determine the ash percentage. According to Aksnes and Opstvedt (1998), the carbohydrate content was calculated as following: Carbohydrate (%) = 100 − (Protein + Lipid + Ash + Moisture). All the above-mentioned Laboratory tests for sediment and algae were conducted in the Laboratory of National Institute of Oceanography and Fisheries, NIOF, Egypt.

Modeling program for Algal lipid production

Based on experimental findings from the cultivation of Spirulina (*Arthrospira platensis*), this dashboard displays visualizations of algae biofuel production. By integrating variables like water type, cultivation time, sediment concentration, and experimental setup, as well as a Python-based model was developed to forecast biodiesel yield and lipid production. Figure 2 presents the flow chart models incorporate multiple parameters including water media type, sediment concentration, cultivation time, and experimental setup to predict lipid production and biodiesel yield. The model could be used to study the effect of the different parameters on the biodiesel yield.

The model general framework consists of: two water types, which are freshwater (7.96% lipid at 18 days) versus seawater (5.61% lipid at 18 days), the cultivation period is modeled as linear lipid accumulation at various periods from 1 to 18 days, experimental conditions are field trials which are scheduled to be added to the laboratory Setup, and density correction is 0.85 kg/l which is of the algal oil density. Important required operational parameters include a 20% methanol of oil weight, a 10% lipid reduction for every added 10 g/l/day (1%) sediment concentration, and a 1% of oil weight required for caustic soda. Comprehensive parameter control is made possible by this dynamic program, which makes it possible to accurately predict lipid yield and biodiesel output under various cultivation conditions.

Statistical analysis

All experimental cultivations and biochemical assays were conducted in triplicate to guarantee reproducibility and data integrity. The results are shown as the mean value (±) the standard deviation (STDEV) to show how precise the measurements were for different environmental factors, such as the type of media and the concentration of sediment. In particular, the differences between replicates for the amounts of protein, lipid, ash, and carbohydrates were analyzed, and

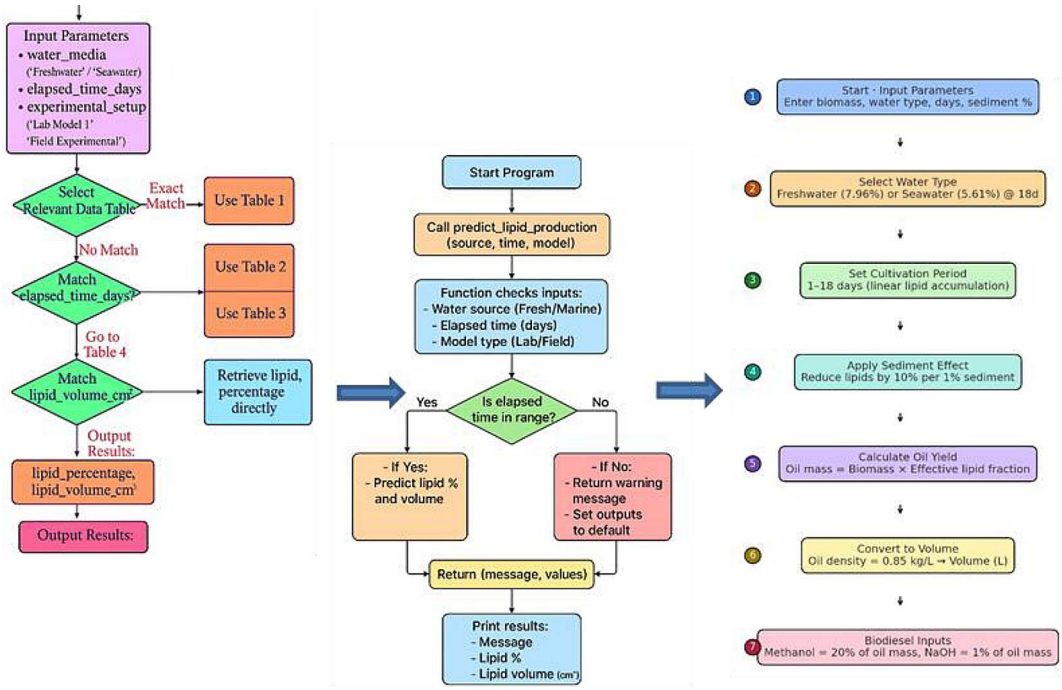

Figure 2. Display the models incorporate multiple parameters including water media type, sediment concentration, cultivation time, and experimental setup to predict lipid production and biodiesel yield

the standard deviations were always kept at or below 0.20%. A modeling program was also used to find the best conditions for growing the maximum lipid yield.

Moreover, the data was examined with IBM SPSS. An independent sample t-test was conducted to compare the lipid yields of different types of media. To check for significant estimation bias, Pearson's correlation coefficient (r) was used to see how strong the relationship was between predicted and observed values and paired t-tests. The p-value for statistical significance was set at less than 0.05. To determine how production has changed over time (1-18 days), regression slopes were used.

RESULTS AND DISCUSSION

Weather condition under lab and field during growth of algae

At 8:00 AM, 12:00, and 17:00 during algal growth period, the laboratory temperature readings were 20 °C, 25 °C, and 23 °C, with corresponding relative humidity values of 67%, 50%, and 61%. Temperatures in the field at the same times were 32 °C, 37 °C, and 40 °C, and relative humidity was 63%, 30%, and 44% as shown in Figure 3 (A, B and C). The solar radiation mean values were 957, 982, and 977 W m^{-2} and measured at 8:00, 12:00, and 16:00 in the field, respectively. The laboratory's light levels were simultaneously 2673, 1676, and 1072 lux.

Laboratory experiments

After seven days, freshwater cultures supplemented with 10 g/l/day (1%) sediment in the lab model had a lipid content of 6.26%, while seawater cultures had a lipid content of 5.52%. In freshwater, the protein and carbohydrate contents were 54.47% and 22.56%, respectively, while in seawater, they were 28.56% and 49.36%. While protein and carbohydrate composition exhibited similar trends (53.6% and 25.8% in freshwater versus 33.6% and 46.96% in seawater), the second model's lipid content was somewhat lower (5.72% in freshwater and 5.11% in seawater) as shown in Table 1 and Figure 4. While protein content steadily increased as carbohydrate levels decreased, lipid accumulation increased over time in both models. Higher protein and lipid levels were consistently supported by freshwater, whereas higher carbohydrate accumulation was encouraged by seawater. One possible explanation is that salinity stress moved the metabolism process from protein synthesis to carbohydrate storage.

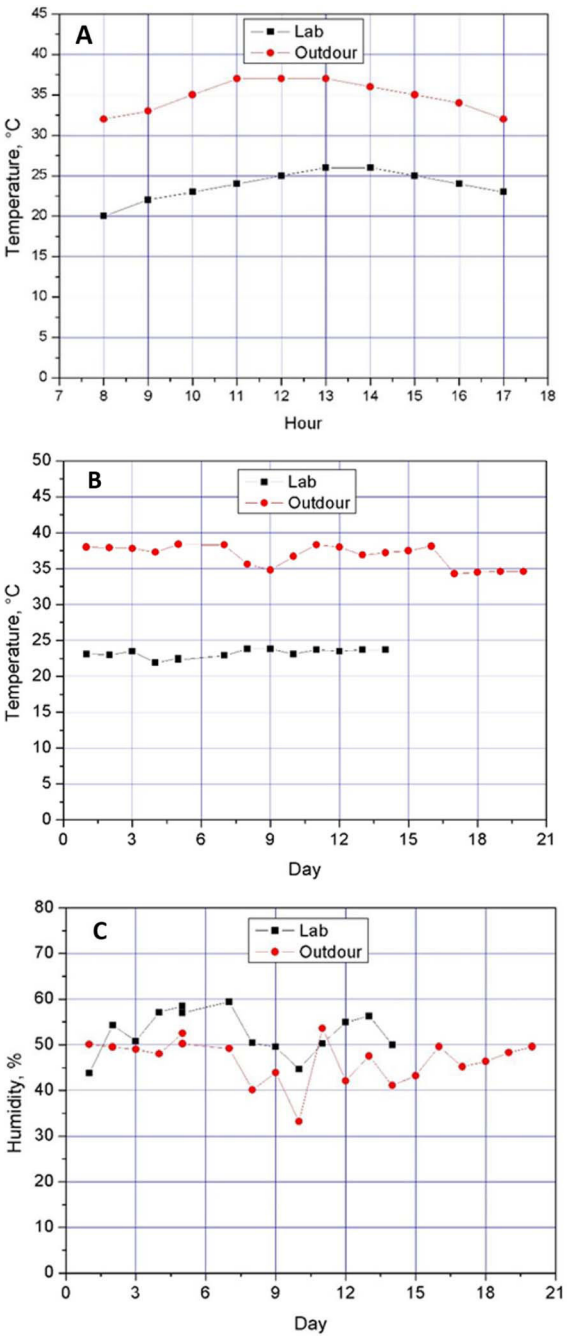

Figure 3. The hourly average changes in temperature (A) and the daily average changes in temperature B) as well as relative humidity (C) for both laboratory and outdoor conditions during the algae lipid production process. Data were recorded from 8:00 AM to 17:00 PM over experimental duration 14 days (lab. conditions) and 20 days (outdoor conditions)

The results in Table 1 show the percentages of lipid oil that Spirulina platensis produced in the lab scale experiment over 4 and 7 days. The experiment tests show that two types of growth media (freshwater and seawater) and two types of light (LED and lamp) affect growth. The experimental data for both media, specifically under LED lighting, were compared with the outputs of mathematical models (Model 1 and Model 2). All experimental groups sustained a biomass concentration 1 g/l and a sedimentation rate of 10 g/l/day to obtain 1% concentration of sediment, whereas control groups produced no lipids. An independent t-test was used to see how well the two media worked under LED lighting. Freshwater media yielded higher lipid percentages (Mean = 6.26%) than seawater (Mean = 5.52%). Freshwater did better, but the difference was not statistically significant at the lab scale ($p = 0.111$). This means that both media are good for short-term lab cultivation. The effect of the light source was assessed after seven days. In freshwater, LED lighting was much better than the lamp, producing an extra 0.53% of lipids. Seawater: LED lighting also caused a 0.39% increase compared to the lamp. For both types of media, LED lighting is better than regular lamp lighting at promoting lipid production.

The correlation analysis results show how well the mathematical models predicted the results of the lab tests. Freshwater (Model 1) was a perfect positive correlation ($r = 1.00$) with the observed LED data, which means that the model perfectly shows how growth changes from day 4 to day 7. Seawater (Model 2) also showed a perfect correlation ($r = 1.00$), which was exactly what was seen in the experiment. From day 4 to day 7, the lipid percentage in both growth media rose steadily. In the lab, the combination of freshwater and LED lighting produced the most lipids overall ($6.26 \pm 0.03\%$).

Field experiment

Under field conditions, lipid production peaked on day 18 with a sediment addition of 10 g/L/day. The highest lipid content in freshwater cultures was 7.96%, while in seawater it was 5.61%. Table 2 shows that the levels of carbohydrates and proteins were different in different media. For example, freshwater had 23.1% and 51.3% of them, while seawater had 27.7% and 44.7%. Longer growing periods in natural sunlight usually lead to more lipids being stored. However, the levels of lipids in seawater reached their highest point on day 16 and then started to go down. This was probably because nutrients were used up, metabolic waste built up, and the water became saltier as it evaporated. The freshwater system, on the other

Table 1. Statistical examination of the biochemical composition of Spirulina grown in various media; juxtaposing laboratory experimental findings with predictive model results at 4-day and 7-day elapsed time

Media	Lighting type	Spirulina Platensis g/l	Sediments g/l/day	Lipids oil % at		Protein %		Ash %		Carbohydrates %	
				4 day	7 day	4 day	7 day	4 day	7 day	4 day	7 day
Control 1 (Freshwater)	LED	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Lamp										
Control 2 (Seawater)	LED	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Lamp										
Freshwater	LED	1	10	5.87 ±0.02	6.26 ±0.03	51.73 ±0.02	54.46 ±0.04	9.56 ±0.03	9.52 ±0.03	26.65 ±0.10	22.86 ±0.06
	Lamp	1	10	**	5.73 ±0.02	**	53.54 ±0.03	**	9.35 ±0.02	**	25.77 ±0.02
Seawater	LED	1	10	5.17 ±0.03	5.52 ±0.01	47.56 ±0.02	49.35 ±0.05	9.53 ±0.02	9.34 ±0.04	29.94 ±0.02	28.55 ±0.02
	Lamp	1	10	**	5.13 ±0.02	**	46.93 ±0.03	**	7.73 ±0.01	**	33.70 ±0.20
Model 1 (freshwater)	-	1	10	5.43	5.87	*	*	*	*	*	*
Model 2 (Seawater)	-	1	10	4.89	5.19	*	*	*	*	*	*

Note: *values were not predicted by the model, **values were not measured.

hand, stayed more stable and supported both biomass growth and lipid synthesis during the whole study. In every model, it is obvious that freshwater produced more proteins and lipids than seawater. Additionally, seawater produced more carbohydrates than freshwater, indicating a metabolic change brought on by stress. Longer cultivation times resulted in an increase in protein and a decrease in carbohydrates, which is consistent with the metabolic change of spirulina under nutrient stress.

Lipids oil production and model validation

The experimental results in Table 2 show the percentage of lipid oil produced by Spirulina platensis in different growth media and over different time periods. The research juxtaposed the empirical data from freshwater and seawater media with mathematical model outputs at 14, 16, and 18 days.

The control groups for both freshwater and seawater showed no sediment buildup (0.0 g/L/

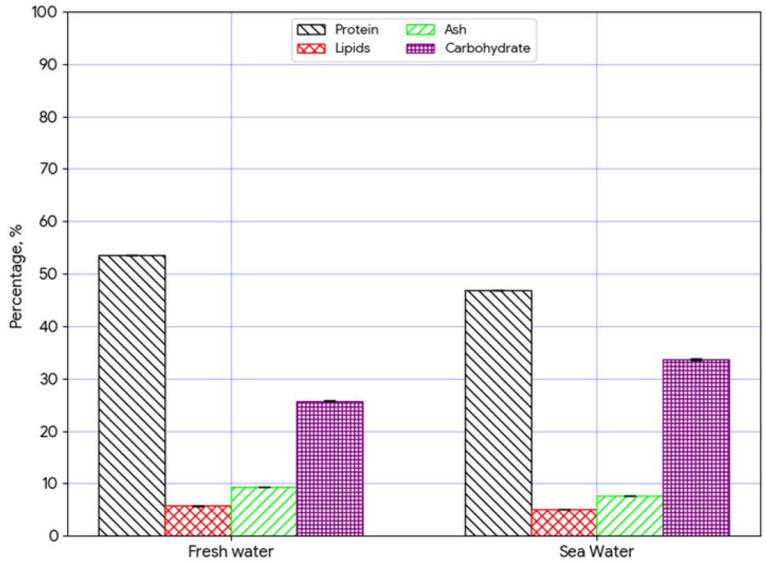

Figure 4. Presence of the lipids, protein, ash and carbohydrate percentage production from algae under laboratory experimental conditions after 7 days

day) and produced no lipid oil during the 18-day period (0.0%). This set a clear baseline for experimental treatments.

The lipid oil percentage in the freshwater system changed over time. It started at 6.10 ± 0.05% on day 14, decreased slightly on day 16, and reached its highest point of 7.96 ± 0.00% on day 18. Model 1 (freshwater) showed a similar pattern assessing values of 5.672% 5.245%, and 7.164% for 14, 16, and 18 days, respectively.

The seawater medium showed a different trend. The highest lipid yield was seen on day 14 (6.26 ±pm 0.53%), and it slowly dropped to 5.64 ±0.04% by day 18. The results for Model 2 (seawater) were similar to those for Model 1, with an estimate of 4.295% on day 14, which went up to 5.368% on day 16 and then stayed the same at 5.110% on day 18. The data shows that both media could support a biomass concentration of 1 g/L and a sediment rate of 10 g/L/day. However, by the end of the 18-day period, the freshwater medium had a higher maximum lipid percentage than the seawater medium. The mathematical models (Models 1 and 2) yielded the estimations that predominantly corresponded with the trends observed under the empirical field conditions, although slight differences were distinguished in the magnitude of the percentages.

Statistical comparison of growth media (Independent T-test)

An independent t-test was conducted on the experimental data collected at all-time points to ascertain whether the type of media (freshwater vs. seawater) significantly affected lipid production. There was no statistically significant difference in lipid oil percentage between freshwater (Mean = 6.52%) and seawater (Mean = 5.88%, t (4) = 0.84, p = 0.446). Freshwater had a higher peak on day 18, but overall, both media performed similarly in the field over the 18-day period.

MODEL VALIDATION AND CORRELATION ANALYSIS

A Pearson correlation and a paired T-test were used to compare the observed and predicted values to see how accurate the mathematical models (Model 1 and Model 2) were.

Model 1 (freshwater) demonstrated an extraordinarily robust and statistically significant correlation with empirical data (r = 0.999, p = 0.012). A paired t-test showed that there was no significant difference between the model and the observed values (p = 0.087). This means that Model 1 is a very good predictor of freshwater conditions. Model 2 (seawater) showed a negative correlation

Table 2. Statistical examination of the biochemical composition of Spirulina grown in various media (freshwater and seawater); juxtaposing Field experimental findings with predictive model results at 14, 16, and 18-day elapsed time

Media	Elapsed time (Days)	Spirulina platensis g/l	Sediments g/l/day	Protein (%)	Lipids (%)	Ash (%)	Carbohydrates (%)
Control 1 (freshwater)	14, 16, 18	1	0.0	0.0	0.0	0.0	0.0
Control 2 (seawater)	14, 16, 18	1	0.0	0.0	0.0	0.0	0.0
Freshwater	14	1	10	55.42±0.73	6.04±0.33	9.52±0.48	22.27±1.01
	16	1	10	58.06±1.14	5.56±0.45	8.89±0.51	20.83±1.98
	18	1	10	51.33±0.89	7.96±0.23	11.01±0.49	23.13±0.85
Seawater	14	1	10	48.25±1.41	4.95±0.52	7.99±0.48	31.46±1.28
	16	1	10	49.43±0.98	5.76±0.43	9.88±0.44	27.71±1.17
	18	1	10	44.73±1.09	5.61±0.74	7.79±0.51	34.59±0.17
Model 1 (freshwater)	14	1	10	*	5.672	*	*
	16	1	10	*	5.245	*	*
	18	1	10	*	7.164	*	*
Model 2 (seawater)	14	1	10	*	4.295	*	*
	16	1	10	*	5.368	*	*
	18	1	10	*	5.110	*	*

Note: * Values were not predicted by model.

(r = -0.927, p = 0.244). Even though the average values were not statistically different (p = 0.199), the model did not show the downward trend that was seen in the seawater field data.

Linear regression was used to find out how quickly lipid production changed over the course of 18 days. Freshwater trendt showed a positive growth rate (Slope = +0.465 per interval) and an R^2 of 0.52. This shows that the amount of lipids is steadily rising as the culture grows. Trend in seawater: There was a negative growth rate (slope = -0.155 per interval) and a high R^2 = 0.86. This indicates that in seawater environments, lipid production reaches its zenith early on and subsequently experiences a consistent decline.

Energy consumption and efficiency

The field setup, which relied on passive solar input, only needed 0.74 W/day, compared to 192 W/day for the first lab model and 120 W/day for the second. However, because of its larger scale and duration, the total field energy consumption was higher (14.2 kWh) than that of lab models 1 and 2 (2.7 kWh and 1.68 kWh, respectively) as shown in Table 3. The field system showed higher efficiency per unit produced despite a higher overall energy demand, demonstrating the viability of solar-integrated, large-scale cultivation.

Modeling results

The model replicated the effects of reactor volume (1–10 m³), water type, cultivation time, and sediment concentration (1–5%) as shown in Table 4. As the amount of sediment increased, lipid production steadily declined, falling from about 5% at 2% sediment to less than 3.5% at 5% as shown in Figure 5. Also, this effect was lessened by larger photo-bioreactors; yields for 1 m³ and 10 m³ freshwater at 1% sediment were 60.4 kg and 608.9 kg, respectively as shown in Figures 6 and 7. On all scales, freshwater performed better than seawater. These patterns demonstrate the

Table 3. Illustrated the power and energy requirement for different conditions field and two lab models and two different water growth media for production of oil from algae

Experiment		Elapsed time, days	Power (W)	Energy (W.h)	Energy (W.h) / day	Energy (kW.h)	Total Energy (kW.h)
Lab	First model (LED)	14	8	8	192	0.192	2.688
	Second model (lamp)	14	5	5	120	0.12	1.68
Field	Fresh water (water pump)	20	373	31.083	746	0.746	14.174
	Sea water (water pump)	20	373	31.083	746	0.746	14.174

Table 4. Illustrates the modeling input parameters, single calculation results, and optimum condition analysis for Spirulina algal lipid production

Category	Parameter	Value
Input parameters	Water volume (m³)	10
Input parameters	Sediment (g/l/day)	10
Input parameters	Water type	Freshwater
Input parameters	Cultivation period (days)	18
Single calculation results	Oil yield (kg)	12178.8
Single calculation results	Methanol needed (kg)	2435.76
Single calculation results	Caustic soda needed (kg)	121.79
Single calculation results	Effective lipid %	7.164
Optimal conditions analysis	Max oil yield (kg)	12178.8
Optimal conditions analysis	Water Volume (m³)	10
Optimal conditions analysis	Sediment (g/l/day)	10
Optimal conditions analysis	Water type	Freshwater
Optimal conditions analysis	Cultivation days	18
Optimal conditions analysis	Methanol (kg)	2435.76

detrimental effects of high sediment loads on nutrient availability and light penetration, which decreased lipid biosynthesis. Growth duration was emphasized as a crucial factor by longer cultivation times, which also raised lipid yields.

Biodiesel additive requirements

The main additive was methanol, which varied from 12.2 to 121.8 kg based on the size of the system, while the amount of NaOH needed was still minimal (0.6–6.08 kg). Because of the higher lipid yields, larger volumes needed more methanol. As the concentration of sediment increased, less lipid was produced, which decreased the need for additives. For instance, the amount of methanol required in a 10 m³ freshwater culture decreased from 121.8 kg at 1% sediment to 67.6 kg at 5% as shown in Figure 8. In addition, NaOH showed a comparable, slightly milder, pattern.

Discussion

The current research study illustrates the production of biodiesel from microalgae under both laboratory and field conditions, with clear variations in biochemical composition and lipid productivity influenced by water type, cultivation duration, and environmental conditions. It is clear that freshwater consistently yielded higher lipid content compared to seawater under all tested conditions. This result agrees with previous studies, which indicated that elevated salinity imposes physiological stress on microalgae, negatively affecting lipid biosynthesis and overall growth performance (Li et al., 2018; Wang et al., 2018). Salinity stress may be able to affect cellular homeostasis and reduce photosynthetic efficiency, therefore limiting lipid production.

Moreover, the increase in lipid content over time in both freshwater and seawater cultures aligns with the established understanding that lipid accumulation typically occurs during the late log exponential and stationary growth phases. During these stages, nutrient limitation particularly nitrogen nutrient depletion induces metabolic shifts toward lipid production (Li et al., 2018). However, the decrease in lipid productivity observed in seawater after 16 days can be attributed to prolonged exposure to salinity stress, nutrient depletion, and possible metabolic imbalances, which collectively inhibit sustained lipid synthesis.

In addition, the lipid biochemical composition of microalgae was significantly different between freshwater and seawater systems. Protein content was observed lower in seawater, while carbohydrate levels were higher. It is indicated that microalgae subjected to saline conditions tend to reallocate their metabolic pathways toward carbohydrate production as opposed to protein synthesis, as a stress adaptation mechanism (Singh et al., 2024).

As well as, the field experiment illustrated the performance of freshwater systems, with the maximum lipid yield was found at 18 days. This confirms the hypothesis that optimizing cultivation time is critical for maximizing biodiesel production. Importantly, the energy consumption

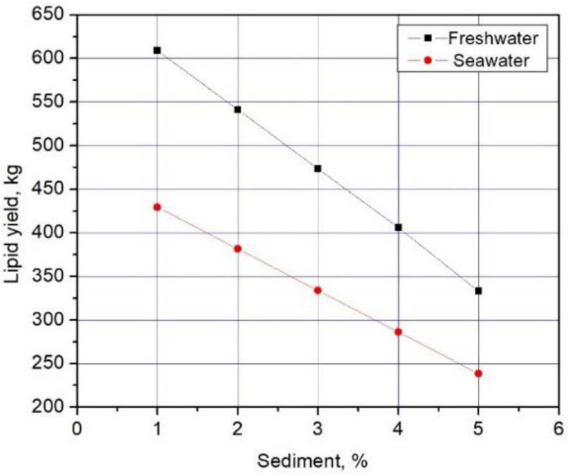

Figure 5. Illustrate the effect of sediment percentage on the lipid produced for freshwater and seawater in 10 m³ water volume photo-bioreactor by using the modeling program

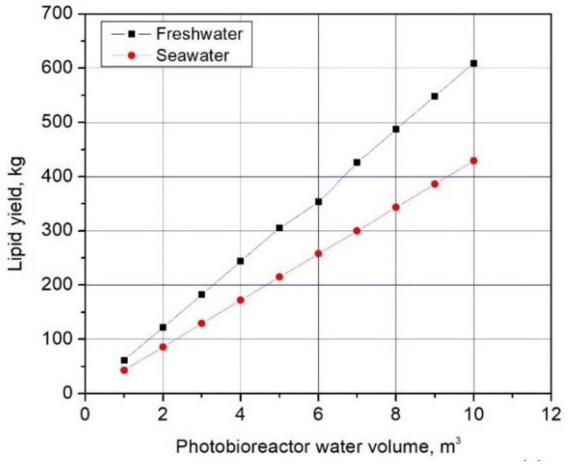

Figure 6. The algal lipid production for both freshwater and seawater at 1% sediment after 18 days' cultivation period in modeling program

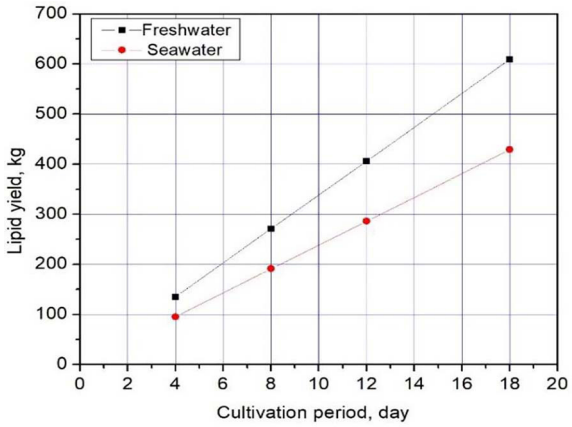

Figure 7. The effect of algal cultivation period days on lipid oil production in 10 m^3 water volume, 1% sediment, freshwater and seawater by using the modeling program

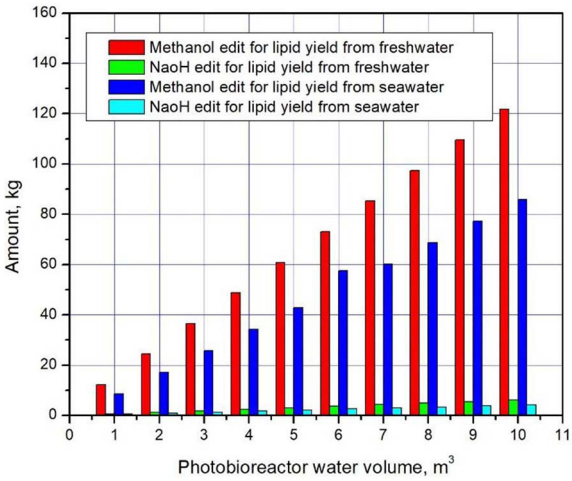

Figure 8. The effect of algal cultivation period days on lipid oil production for freshwater and seawater and different water volume at 1% sediment

analysis demonstrated that field conditions required significantly lower power input than laboratory systems. This finding is consistent with previous research indicating that large-scale outdoor cultivation systems, such as open ponds, are more energy-efficient and economically feasible than controlled laboratory setups (Shirvani, 2012). The results indicated that freshwater provides a more favorable environment for lipid production in microalgae. Also, cultivation time and environmental conditions are very important in determining both biochemical composition and overall productivity. Future research should focus on improving lipid yields in seawater systems through such strategies as nutrient optimization or the use of salt-tolerant algal strains.

CONCLUSIONS

The conducted study shows that Spirulina platensis could be more productive and stable in freshwater media than seawater, leading to higher lipid and protein content. Photovoltaic-powered photo-bioreactors integrated under outdoor conditions decreased the external energy inputs without influencing the biomass productivity. 14.2 kWh of solar energy were consumed by the set-up in comparison to 2.7 kWh and 1.7 kWh of the laboratory models. In freshwater, longer cultivation time increased lipid and protein yields, but in seawater cultures, the yields decreased sharply after day 14.

Statistical analysis showed that freshwater media yielded higher lipid levels than seawater ($p < 0.05$) and showed better long-term stability and growth patterns under LED illumination. Moreover, the predictive ability of Model 1 was high in both experimental environments, with a correlation coefficient of $r > 0.999$. Furthermore, the successful application of the dredged sediment from Lake Burullus as a natural nutrient amendment provides a viable and sustainable substrate for the growth of algal biomass. Future work should be directed to scaling up automated nutrient delivery and to mitigating salinity stress in seawater-based systems to boost marine-based biodiesel production.

In conclusion, this work underlines the importance of taking into account productivity and lipid percentage to correctly assess the bioenergy potential of microalgae. Although improved experimental setup (specific illumination, mixing and 10 g/L/day sediment dosing) was informative, acknowledge some limitations, such as the absence of sediment-free controls need to be acknowledged. Future studies will build upon the current findings by adding detailed FAME profiles and biodiesel quality parameters, such as cetane numbers and iodine values, to completely determine the commercial viability of the produced biofuel.

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