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Urban photobioreactor for CO_2 sequestration and microalgal biomass production

Fotobiorreactor urbano para el secuestro de CO_2 y la producción de biomasa microalgal

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Abstract

The growth system of microalgae photobioreactors (PBRs) has drawn a lot of interest as a viable and sustainable method for generating quality biomass for value-added products and biofuels. The objective of this research work is to cultivate microalgae species *Chlorella vulgaris* in a photobioreactor that was designed, fabricated, and powered by solar energy system. Three experimental conditions were compared with 1:4 ratios of microalgae culture (40L) and fresh water (10L) having 100mL of media (nutrients) used in each experiment with control sample (ambient air aeration) experiment # 1, injecting 200 g of CO_2 for 15 sec (experiment # 2), and 300g of CO_2 for 25 sec (experiment # 3) on alternate days during the cultivation period. All experiments showed the reduction of nutrients concentration (orthophosphate and nitrate) and enhancement of biomass productivity with respect to 10 days of cultivation period. Experiments 1, 2 and 3 showed removal of orthophosphate as 50%, 41.74% and 60.78% respectively, whereas nitrate removal was 22%, 48% and 58%. Biomass productivity from experiments 1, 2 and 3 after 10 days of cultivation period were 196.63 mg/L, 203.43 mg/L, 318.76 mg/L respectively. Statistical analysis revealed that supplying CO_2 from external source in experiment # 2 and experiment # 3 have same pattern of statistical significance with co-relationship between two groups of means with p-value of 6.306×10^{-14}. The maximum microalgal biomass was recovered from experiment # 3, with 7.98% by weight protein content yield and lipid content yield 37.4% by weight (1.87/ 5 g of dried biomass). Kinetic study showed volumetric mass transfer capacities of KO_2 and KCO_2 were found to be $1.763\times10^{-7}m^3/s$ and $1.676\times10^{-7}m^3/s$, with better result of KCO_2 gas transfer capacity of the system. In the extracted lipids favorable qualities of fatty acids for the production of microalgae biodiesel were found such as myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:1), and linolenic acids (C18:3). The use of urban microalgae photobioreactors is an environmentally sustainable strategy that can contribute significantly to the bio-based economy and reduce the negative effects of traditional fossil fuel usage on the environment.

Keywords: Carbon sequestration, Urban photobioreactor, Carbon capture, Biomass productivity, CO_2 bio-fixation, *Chlorella vulgaris*, Biofuel production.

Resumen

El sistema de crecimiento de fotobiorreactores (PBRs) de microalgas es de gran interés pues es un método viable y sostenible para generar biomasa de calidad destinada a productos de valor agregado y biocombustibles. En este estudio se cultivó la especie de microalga *Chlorella vulgaris* en un fotobiorreactor diseñado, fabricado y alimentado por un sistema de energía solar. Se compararon tres condiciones experimentales con proporciones de 1:4 de cultivo de microalgas (40 L) y agua fresca (10 L), utilizando 100 mL de medio (nutrientes) en cada experimento: un experimento control (aireación con aire ambiente, experimento # 1), la inyección de 200 g de CO_2 durante 15 segundos (experimento # 2), y la inyección de 300 g de CO_2 durante 25 segundos (experimento # 3) en días alternos durante el periodo de cultivo. Todos los experimentos mostraron una reducción en la concentración de nutrientes (ortofosfato y nitrato) y un aumento en la productividad de biomasa tras un periodo de cultivo de 10 días. Los experimentos 1, 2 y 3 mostraron remociones de ortofosfato del 50 %, 41,74 % y 60,78 %, respectivamente, mientras que la remoción de nitrato fue del 22 %, 48 % y 58 %. La productividad de biomasa en los experimentos 1, 2 y 3 tras 10 días de cultivo fue de 196,63 mg/L, 203,43 mg/L y 318,76 mg/L, respectivamente. El análisis estadístico reveló que el suministro de CO_2 desde una fuente externa en los experimentos # 2 y # 3 sigue un patrón similar de significancia estadística, con una correlación entre ambos grupos de medias y un valor de p de $6\,306 \times 10^{-14}$. La mayor biomasa de microalgas fue recuperada del experimento # 3, con un contenido proteico del 7,98 % en peso y un contenido lipídico del 37,4 % en peso (1,87 g/5 g de biomasa seca). El estudio cinético mostró que las capacidades de transferencia volumétrica de masa de KO_2 y KCO_2 fueron de $1\,763 \times 10^{-7}$ m^3/s y $1\,676 \times 10^{-7}$ m^3/s, respectivamente, siendo más eficiente la capacidad de transferencia de KCO_2 del sistema. Los lípidos extraídos presentaron ácidos grasos favorables para la producción de biodiésel de microalgas, como ácido mirístico (C14:0), palmítico (C16:0), palmitoleico (C16:1), oleico (C18:1), linoleico (C18:1) y linolénico (C18:3). El uso de fotobiorreactores urbanos de microalgas es una estrategia ambientalmente sostenible que puede contribuir significativamente a la economía basada en recursos biológicos y reducir los efectos negativos del uso tradicional de combustibles fósiles sobre el medio ambiente.

Palabras clave: Secuestro de carbono, Fotobiorreactor urbano, Captura de carbono, Productividad de biomasa, Biofijación de CO_2, *Chlorella vulgaris*, Producción de biocombustibles.

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1 Introduction

With over 75% of all greenhouse gas emissions and almost 90% of all carbon dioxide emissions coming from combustion of fossil fuels like coal, oil, and natural gas are by far the biggest cause of climate change. Greenhouse gas emissions cover the Earth, trapping heat from the sun, thus causing climate change and global warming. The world is now warming faster than at any point in recorded history. Over time, rising temperatures are altering weather patterns and upsetting the natural equilibrium of the environment. This poses many risks to human beings and all other forms of life on Earth (United Nations, 2024). Between 1900 and 2020, the Earth's atmosphere saw a temperature increase of around 1.1 °C, leading to changes in weather patterns and global warming, the global average temperature rises to 2 °C above the level of pre-industrial times, whilst pursuing efforts to limit the increase to 1.5 °C, was the stated objective in the Paris Agreement. This will also help to achieve the UN Sustainable Development Goal (SDG) goal 13 i.e., to combat climate action formulated in 2016.

The world's energy consumption is mostly fueled by fossil fuels, which account for around 85% of all energy sources worldwide. Scientist have issued warnings about the risk of running out of finite fossil fuel supplies without creating a viable alternative energy source to take the place of the declining oil reserves. Another issue that has not received enough attention in many oil-producing nations is the pollution and emissions that come from the exploration and production of fossil fuels (Valavanidis, 2023). The challenges of depleting fossil fuel reserves and environmental crisis emanating from the use of fossil fuels, it is therefore increasingly necessary to find environmentally sustainable and clean fuels for future uses to mitigate climate change and global warming (Mahapatra et al., 2021; Rodionova et al., 2017). In order to reduce the impact of GHG's emissions from burning of fossil fuels, biofuels are produced directly or by biological processes or obtained via the chemical conversion of biomass as a replacement to fossil fuels (Rodionova et al., 2017). There are various types of biofuels, for example, first-generation biofuels consist of ethanol derived from starch-rich food crops or biodiesel created from residual animal fats like frying grease. The second generation is composed of bioethanol derived from non-food cellulosic material and biodiesel produced from oil-rich plant seeds like pongamia or jatropha. The most promising method to fulfill the world's energy demands is the third generation of biofuels, which are produced from microalgae, cyanobacteria, and other microorganisms (Rodionova et al., 2017).

The threat posed by air pollution causing climate change brought on by numerous human activities has gained attention from all around the world. Though formerly thought to be a promising technological solution to lessen this alarming situation, carbon capture and storage (CCS) techniques are now regarded as not economically feasible, and it is uncertain what effect they will have on the environment in the future (Sievert et al., 2023). As an alternative, the use of microalgae for the biological capture of carbon dioxide (CO_2) is seen to be a promising method for recycling the surplus CO_2 produced by vehicles, power plants, industries, volcanic eruptions, the breakdown of organic materials, and forest fires. Moreover, CO_2 can be taken up by microalgae and regenerated into biomass, which can then be used as a carbon source to make lipids for the synthesis of bioenergy and other products with added value (Sievert et al., 2023). The mass scale biomass can be achieved by microalgae cultivation, using two major cultivation systems such as open raceway pond and a photobioreactor system. The higher biomass productivity can be obtained through controlled environment using a photobioreactor. Considering the microalgae metabolism, it can be classified as photoautotrophic, photoheterotrophic, heterotrophic and mixotrophic. Sunlight, some basic inorganic elements like carbon dioxide (CO_2), and metal salts are necessary for the cell development or cultivation metabolism process of microalgae. Heterotrophic microalgae, on the other hand, require an additional source of certain organic compounds and nutrients, such as nitrogen (N) and phosphorus (P). Previous research demonstrates that growing Chlorella on a mixotrophic medium increases photosynthesis efficiency and provides exogenous organic resources. Furthermore, throughout the culture process, microalgal farming significantly lowers greenhouse gas (CO_2) emissions in addition to producing biomass and biofuels. On the other hand, wastewater from different sources can be used as a culture medium for microalgae. Microalgal strains that are photohetero-

trophic, mixotrophic, or heterotrophic have grown in both light and dark media (Saratale et al., 2022).

For the cultivation of microalgae, photo bioreactor (PBR) is used having enclosed culture vessel with adjustable operational settings to regulate biomass. As a photosynthetic organism, microalgae can be grown in both closed (photobioreactor) and open (pond) systems. Better control over culture settings is possible with photobioreactors, which are being developed for maximum productivity, economic effectiveness, and minimum maintenance. The microalgae growth, photosynthesis, and lipid accumulation are dependent on a number of variables, including light, temperature, the medium's pH, the presence of CO_2, and macronutrients including potassium, phosphates, and nitrates (Saratale et al., 2022).

An urban photobioreactor is an alternative approach to greening that was created for urban environment where traditional greening is impractical because of space constraints, land values, and air pollution. It is based on the high CO_2 fixation and O_2 production efficiency of microalgae and photobioreactor technology. It is equal to one mature tree or 200 square meters of lawn, depending on the rate of carbon fixation. Because of its multipurpose nature, thoughtful design, and secure construction, it blends in with urban surroundings (Rehman et al., 2022). The microalgae cultivation in a photobioreactor includes number of factors such as microalgae species, photobioreactor design, choice of light source, mixing mechanisms, nutrient supply, temperature and pH control, and considerations for harvesting and processing. Emphasizing a high surface area to volume ratio is important in this design process. Given the light-dependent nature of the reaction, a greater surface area facilitates enhanced light penetration, which is fundamental requirement for photosynthesis (Stojiljković and Spasojević, 2023).

One sustainable method for capturing carbon dioxide and utilizing it to produce renewable products and reduce emissions is the use of photosynthetic microalgae. Past research study has mostly concentrated on the byproducts of microalgae, especially biofuels, rather than their capacity to sequester CO_2. *Chlorella sp.* was grown in an investigation at CO2 concentrations similar to those seen in the fuel gases from power plants. It was discovered that 5% de CO_2 was the ideal concentration for the production of microalgae biomass. The cultures' CO_2 removal efficiency was then continuously observed using a nondispersive infrared sensor. Over the course of 14 days, the average CO_2 removal effectiveness was 17.5%, which is significantly greater than the values reported in the literature when no direct real-time monitoring system is used (Scheufele et al., 2019). Microalgae have the ability to produce 10–20 times as much oil as vegetable oil seed crops, they are regarded as a promising feedstock for the production of third-generation biofuels (Leflay et al., 2021).

The cleaning of flue gas has generated a lot of interest because of the growing concerns about CO_2 emissions and environmental degradation. One method of reducing CO_2 emissions from flue gas that is seen to be promising is the use of microalgae for photosynthesis. On the other hand, the flue gas pollutants might prevent microalgal growth, which would reduce the rate of CO_2 fixation by microalgae. A steady pH level can help mitigate the inhibitory effects of SO_x, which contribute to the low pH, while NO_x can be used as a source of nitrogen to encourage the growth of microalgae once it dissolves and oxidizes in the culture medium. Fixing CO_2 from flue gas and using NO_x and SO_x as nutrients would generate microalgal biomass, which may be used as a suitable feedstock to make biofuels and bio-based chemicals (Ali et al., 2021). Microalgae can be used as a sustainable feedstock for the production of biodiesel by lipid extraction and a transesterification process because of their high lipid content and rapid growth rates. However, a number of obstacles need to be removed in order to produce biodiesel derived from microalgae, such as high production costs, low lipid productivity, and issues associated with large-scale growth and harvesting. The production of biodiesel from microalgae appears to have a promising future with possible uses in a number of sectors, including agriculture, energy, and transportation (Yen et al., 2015).

The current research study objectives are to design and fabricate a 250 L urban photobioreactor operated by solar energy system to cultivate *Chlorella vulgaris* in freshwater condition. The experiments included the control sample provided with ambient air aeration, while two experiments were conducted

with introduction of intermittent CO_2 concentrations to investigate the influence of CO_2 on biomass productivity and its bio-fixation. On the alternate day basis measurements of the microalgae cultivation growth parameters were monitored and measured such as removal of nutrients, biomass productivity and CO_2 sequestration.

2 Materials and Methods

2.1 Microalgae culture and nutrient medium

The microalgae culture of *Chlorella vulgaris* was obtained from the Marine Resource Department, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Karachi, while the culture media (F/2) Guillards was prepared per liter having composition including 34g Sea salt, 84.15 mg $NaNO_3$, 6 mg $Na_2MoO_4.2H_2O$, 2.9 mg $FeCl_3.6H_2O$, 10 mg $Na_2EDTA.2H_2O$, 33 mg $Na_2SiO_3.9H_2O$, 1.96 mg $CuSO_4.5H_2O$, 4.4 mg $ZnSO_4.7H_2O$, 1.26 mg $Ma_2MoO_4.2H_2O$, 36 mg $MnCl_2.4H_2O$, 2 mg $CoCl_2.6H_2O$, 0.4 mg Vitamin B1, 0.002 mg Vitamin B12, 0.1 mg Biotin.

2.2 Designing of a photobioreactor

A photobioreactor for microalgae cultivation includes number of factors such as microalgae species, photobioreactor design, choice of light source, mixing mechanisms, nutrient supply, temperature and pH control, and considerations for harvesting and processing. In this design procedure, a high surface area to volume ratio is a critical factor. Given the light-dependent nature of the reaction, a greater surface area facilitates enhanced light penetration, which is fundamental requirement for photosynthesis. To achieve an optimal balance, the surface area to volume ratio can be heightened by employing geometrically efficient designs and configurations. This emphasis on optimizing the surface area to volume ratio underscores the significance of efficient light exposure and nutrient distribution. For a rectangular photobioreactor, based on its geometric specification, the surface area and volume can be calculated by the following equations (1) and (2):

$$A_s = 2HW + 2HL \tag{1}$$

$$V = HWL \tag{2}$$

Where W is the width, H is the height and L is the length of the flat panel photobioreactor. According to Equation 3, the bubble rise rate u_b and the mean bubble diameter d_b determine the shear rate of bubble cultures, or y-aeration. The velocity of bubbles depends on their size and the fluid's characteristics, therefore for medium-sized bubbles in water, 0.24 m/s is an acceptable value obtained by Hadamard–Rybczynski equation The bubble diameter can be estimated based on the fluid properties and conditions using the Calderbank Equation (Gaurav et al., 2024). This equation indicates that the smaller the bubble size, the larger the shear rate and the resulting damage to the microalgae cultures.

$$Y_{aeration} = \frac{2 \cdot u_b}{d_b} \tag{3}$$

Figure 1 shows the schematic diagram of an urban photobioreactor.

2.3 Experimental conditions of cultivating microalgae in a photobioreactor

A rectangular glass photobioreactor having dimensions height 90cm (35.43 inches), width 32cm (12.59 inches), length 121cm (47.63 inches) was designed with volume capacity of approximately $\sim$ 350 L, while its top cover is 97 cm (38.18 inches) and 178 cm (70 inches) having 500 W solar PV panel with size 58 inches $\times$ 26 inches $\times$ 1.5 inches (length $\times$ width and height). The solar PV panel was positioned at an angle of 30° on the top of the photobioreactor assembly to harnesses solar energy efficiently but also aligns with contemporary ecofriendly practices, contributing to a reduced carbon footprint. The photobioreactor was provided with a metallic frame for housing a battery, inverter, and an air pump. For microalgae species *Chlorella vulgaris* light source for photosynthesis being an essential component of the system; two LED of 7 W each operated by solar energy system were installed. The photobioreactor uses a combination of sunlight during the daytime and LED lights during the nighttime obtained from using solar energy obtained through a solar energy system. To maintain even dispersion of light and nutrients, air diffusers are provided for adequate aeration from the bottom of the photobioreactor.

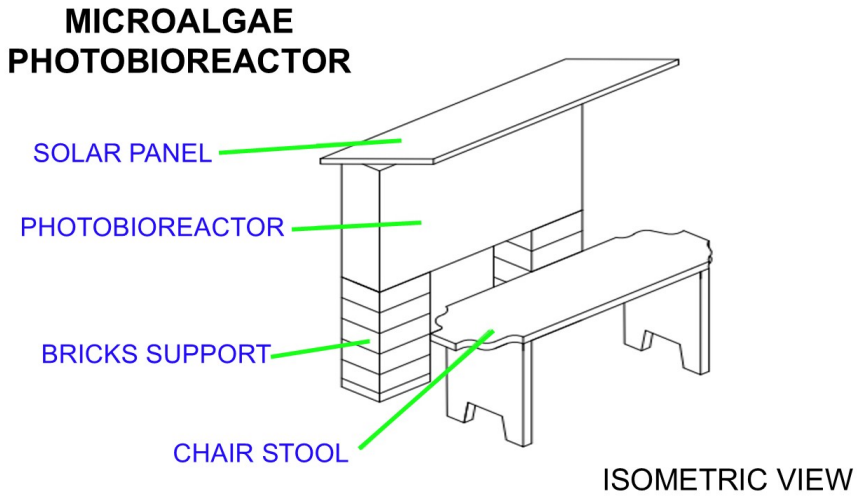

Figure 1. Showing an isometric view of design of an urban photobioreactor.

The microalgae cultivation had a working volume of 100 L. The nutrient (F/2 standard medium) were used as a growth additive for the experiments. The microalgae culture was kept under solar assisted light for 10 days cultivation time. The falling sunlight irradiance (Lux) on the photobioreactor and the solar PV panel and LEDs during the night were measured with a light intensity meter (MS6612T, Mastech, China). Each experiment with different ratios of culture, media (nutrients) and water with/ without carbon dioxide (CO_2), the parameters such as pH, TDS, temperature, and EC were measured using a pH/ORP/EC/TDS/temperature meter (EZ-9910, Multifunction, China).

Three experiments were conducted as per the following protocol by varying the concentration of carbon dioxide in the microalgae culture and water (see Table 1). Carbon dioxide (CO_2) with 98% purity concentration was dosed intermittently on alternate days. The mixing is achieved using air diffusers operated by an air pump (1780 GPH, Kulife Aquarium Air Pump, China), which supplies air in alternate hour for 15 min duration, powered by solar energy system. The microalgae cultivation with air aeration and with CO_2 (200 g y 300 g) enrichment (200 g and 300 g) of intermittent CO_2 injection were used in the subsequent runs of the experiment. This study involves three sets of experiments to investigate the growth of microalgae at various CO_2 concentration levels.

2.4 CO_2 bio-fixation by microalgae during cultivation

The flow rate of CO_2 introduced into the photobioreactor was measured using the continuity equation (4):

$$Q = A \times V \tag{4}$$

Where flow rate (Q), area of the pipe (A), and velocity of CO_2 gas (V) injected into the system through a rubber hose pipe having diameter (0.018 m) used to inject CO_2 in the photobioreactor was measured by using a vernier caliper through which the area was calculated. While the velocity of CO_2 injected into the photobioreactor tank was measured using a Weather Meter (Kestrel 4000NV, USA). The CO_2 was dosed for 15 sec in Experiment # 1 and 25 sec in (Experiment # 2) on alternate days. The volume of CO_2 injected is calculated by multiplying flow rate with dosing time, then the mass of CO_2 is computed by Density= Mass/ Volume.

The use of photosynthetic microalgae for carbon capture offers the potential for a sustainable capture system, which can both reduce emissions and produce renewable products (Borowitzka, 1999). The bio-fixation rate of carbon, R_{CO_2} (g_{CO_2} /L/ day) is calculated using the following mathematical equation (5):

$$R_{CO_2} = \%C \times PB \left(\frac{MW_{CO_2}}{MW_C} \right) \tag{5}$$

Where, R_{CO_2} refer to CO_2 bio-fixation rate,% C indicate total carbon content, PB is the biomass productivity (mg of biomass produced per L per day), MW_{CO_2} denote the molecular weight of CO_2 and MW_C is molecular weight of carbon.%C is the carbon content of the dry biomass, assumed at $\sim$ 50 (Borowitzka, 1999).

2.5 Modelling of carbon dioxide (CO_2) volumetric mass transfer capacity in a photobioreactor

Mass transfer in a photobioreactor is the flow of substances between the gas and liquid phases, including gases (such carbon dioxide and oxygen) and nutrients. Mass transfer is greatly aided by aeration, which is usually accomplished by bubbling ambient air through the liquid medium. The interfacial area between the liquid and gas phases is increased as a result. The film theory is a common method that is used to explain mass transfer at the gas-liquid interface surface (Amaral et al., 2019). This hypothesis states that mass transfer takes place through a thin liquid coating that forms around gas bubbles. The rate at which a substance transitions from the gas phase to the liquid phase is measured by the mass transfer coefficient (k) in Equation 6. Its definition is the relationship between the thickness of the boundary layer (δ) at the gas-liquid interface and the substance's diffusion coefficient (D) in a liquid medium. The mass transfer coefficient for O_2 in photobioreactor is given by (Faruque et al., 2021).

$$k = \frac{D}{\delta} \tag{6}$$

By taking into account the impact of photobioreactor dimensions on mass transfer rates by including its surface area in the calculations. The photobioreactor's surface area directly impacts mass transfer rates because it determines the gas-liquid interface available for gas exchange. Considering the unique geometry of the reactor, the computed volumetric mass transfer capacity shows the rates at which molecules of carbon dioxide and oxygen can move from the gas phase to the liquid phase per unit area of the gas-liquid interface. This is calculated by the Equation 7 (Faruque et al., 2021):

$$k = \frac{D \times A}{\delta} \tag{7}$$

Surface area of a rectangular photobioreactor was calculated by the Equation 8:

$$A = 2\left(L \times W + L \times H + W \times H\right) \tag{8}$$

2.6 Measurement of microalgae growth parameters

2.6.1 Solar irradiance and Temperature

The falling solar irradiance (Lux) on the photobioreactor and LEDs during the night time were measured with a light intensity meter (MS6612T, Mastech, China).

Table 1. Water to media (nutrient) ratios utilized in the experimental study.

	Experiment # 1*	**Experiment # 2***	**Experiment # 3***
Water (L)	40	40	40
Microalgae culture (L)	10	10	10
F/2 standard nutrient media solution (mL)	100	100	100
Intermittent CO_2 injection time	0	15 sec (200 g of CO_2)	25 sec (300 g of CO_2)

*Each experiment was repeated twice, and the results of physical and chemical parameters are presented as an average value.

2.6.2 pH, TDS, TSS and Electrical conductivity

pH, TDS, TSS and electrical conductivity were by multi-functional meter (EZ-9910, Multifunction, China).

2.6.3 Nutrients removal (nitrates and orthophosphate)

Nitrate and orthophosphate concentrations were measured using a DR-500 UV-Vis spectrophotometer (Hach, USA). The nitrate amount was measured by adding powdered aluminum nitrate (Hach, USA) as a reagent to 10 mL of the sample to detect nitrate concentration. In order to measure the amount of nitrate at 425 nm, 10 mL of the prepared solution was introduced to the sample cell, similar to how samples were added and properly mixed by shaking with 8 mL of reagent solution combined with conventional procedures for testing water and wastewater in accordance with the proportions given for the ascorbic acid method (4500-P. E) to determine the concentration of orthophosphate (Amaral et al., 2019). A sample of 10 mL of the solution was added to the spectrophotometer's sample cell after 10 min, and the concentration was determined at 880 nm.

2.6.4 Biomass productivity

The biomass productivity was measured with a UV-Vis spectrophotometer (DR 5000, Hach, USA) at 680 nm from Hach (USA). According to the literature (Leflay et al., 2021), microalgae biomass yields were assessed alternately over the course of a 10-day growing period by measuring the optical density. *Chlorella vulgaris* standard dried biomass was used to plot the standard plot between the known concentration of microalgal biomass (mg/mL) vs absorbance at 680 nm.

2.7 Protein Extraction

The liquid sample with the highest biomass productivity was selected for protein detection and fatty acid analysis. For the protein detection, Lowry standard method was used to the extraction and quantification of proteins. Protein extraction analysis was conducted at the Food and Marine Resources Research Centre, PCSIR Laboratories, Karachi.

2.8 Lipid Extraction and fatty acids compositional analysis

A sample of 250 mL cultivated microalgae suspension was centrifuged at 3000 rpm using multipurpose centrifuge (1580 R, Lab-Tech, Italy) for 30 min to separate the liquid phase from the organic biomass content. Organic biomass was dried at 80 °C for 3 hrs in an oven (YCO-NO1, Gemmy Industrial corporation, Taiwan), after drying the dried biomass chips were pulverized in a mortar and pestle. The Bligh and Dyer procedure was followed to extract lipids from the dried microalgae biomass as per literature using n-hexane as an extraction organic solvent (Leflay et al., 2021) and extracted lipid yield by weight% was measured by weight using an electronic balance (AB 304-S, Mettler Tolendo, Switzerland).

3 Results and Discussion

3.1 Fabricated urban photobioreactor

An urban photobioreactor has been locally designed and fabricated, with dimensions height × width × length (35.43 × 12.59 × 47.63) inches, having total volume of 350 L showcasing an innovative structure tailored for efficient microalgae cultivation for climate change mitigation as depicted in Figure 2.

3.2 Analysis of microalgae growth parameters

3.2.1 Solar irradiance and temperature

In-depth research indicates that higher temperatures can enhance enzymatic activities involved in nutrient assimilation and lipid accumulation, crucial for biofuel production. However, there is an optimal temperature range for each algae species as excessively high temperatures might lead to thermal stress, disrupting cellular functions and impeding growth. Understanding and controlling temperature conditions are crucial for optimizing algae-based biofuel production systems. The results of experiment # 1 showed solar irradiation and temperature profile for the 10 days of cultivation time, measured average values 22.02 °C and 362.5 W/m^2 respectively (see Table 2).

Tables 3 and 4 show variation in temperature during the cultivation period between 22.3 to 24.0 °C and 23.9 to 25.3 °C for experiment # 2 and experiment # 3 respectively. The average solar irradiation measured for experiment # 1 and experiment # 2 were 391.9 and 430.4 W/m^2 respectively. Table 4 suggests that the optimal temperature range for higher biomass production lies between 24 to 25 °C, while solar irradiation of 430.4 W/m^2 yielding maximum 319.9 mg/L of microalgal biomass yield after 10 days of cultivation period.

Figure 2. Locally designed and fabricated urban photobioreactor.

3.2.2 pH, TDS, TSS and electrical conductivity

Algal growth and metabolism are influenced by pH level for both the performance of photobioreactors and the growth of microalgae. It plays a crucial role in determining the availability of nutrients, regulating metabolic activities, and overall health of microalgae within the growing medium (Shuler and Kargi, 2002). In addition to being dependent on CO_2 solubility, the pH value of the culture media appears to be influenced by nitrogen uptake, which is necessary for the development of algal cells and the subsequent consumption of nitrate by microalgae (Borowitzka, 1999).

The pH of the medium for experiment # 1 were between 7.9 to 8.3 giving an average of 8.12 which is greater than the desired value according to the literature (APHA, 2005), resulting in lesser biomass productivity. Whereas the average pH for the course of cultivation was determined to be 7.57 in experiments # 2 and 3 and were found in accordance with previous literature (APHA, 2005).

The growth medium's levels of electrical conductivity (EC) and total dissolved solids (TDS) are crucial in controlling the availability and solubility of essential nutrients, including nitrogen, phosphorus, potassium, and micronutrients. Imbalances in TDS and EC levels, either too high or too low, can impact the nutrient uptake by algae, potentially imposing limitations on their growth. Moreover, elevated TDS and EC levels may subject algae cells to osmotic stress caused due to higher concentration of solutes in the growth medium, differing from the cell's internal environment and affecting water balance and overall cell health.

Table 2 showed decreasing electrical conductivity from 1.34 to 0.98, this is due to EC directly related to the concentration of ions in the medium, including essential nutrients like nitrogen, phosphorus, and micronutrients reduced during the cultivation period. However, adequate nutrient availability is crucial for microalgae growth during its cultivation period (Brindhadevi et al., 2021). A considerable decrease in EC was observed in experiment # 2 and experiment # 3 between 1.13 to 0.82 and 1.34 to 0.95 respectively (see Table 3 and Table 4).

Table 2. Experiment # 1 (ratios used 80% water and 20% microalgae culture).

	Duration of cultivation				
Parameters	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 10**
Temperature (C)	21.8	22.7	21.9	21.3	22.4
Solar irradiation (W/m^2)	365.9	342.6	383	397.1	323.9
pH	8.1	8	8.3	7.9	8.3
EC (μs/cm)	1.34	1.33	1.15	1.11	0.98
TDS (mg/L)	610	600	580	550	540
TSS (mg/L)	71	82	93	98	100
Orthophosphate (mg/L)	104	100	73	60	52
Nitrate (mg/L)	10	9.5	8.8	8.2	7.8
Absorbance (abs)	0.11	0.14	0.16	0.2	0.23
Biomass productivity (mg/L)	113.3	134.4	148.4	176.5	196.63

It has been observed that TDS content decreases drastically in all three experiments # 1, 2, and 3 during cultivation period. The absorbance (light intensity) is found higher in experiment # 3 (with 300g of CO_2 introduced into the photobioreactor), ranging between 0.12 to 0.49 absorbance which subsequently decreases the electrical conductivity of salts in the system. While light intensity itself does not directly contribute to electrical conductivity, but having impact on photosynthesis, biomass growth, and metabolic activities can lead to changes in the ion composition of the culture medium (Morales et al., 2018; Nezammahalleh et al., 2016). Reduced TDS and EC levels can be interpreted as a positive outcome, indicating the utilization of nutrients by the microalgae for their growth and metabolic processes (Nezammahalleh et al., 2016).

The Total Suspended Solids (TSS) demonstrated a notable increasing trend, primarily attributed to the formation of insoluble biomass within the system as presented in Tables 2 to 4. This phenomenon contributes to the heightened turbidity of the system. Importantly, this observed increase in TSS serves not only as a consequence of biomass formation but also as a valuable indicator for quantifying biomass productivity. The rising TSS levels act as a tangible and easily measurable metric, offering a direct means to gauge the effectiveness of biomass production within the photobioreactor cultivation system. The biomass production of microorganisms such as *Chlorella vulgaris* throughout the cultivation phase can be significantly influenced by the levels of total suspended solids (TSS) and total dissolved solids (TDS) in water. During testing, the concentration of TSS increases from 71 to 100 mg/L (experiment 1), 74 to 105 mg/L (experiment 2) and 81 to 110 mg/L (experiment 3), from day one to day tenth during cultivation period. High levels of TSS can affect the light penetration into the water, reducing the availability of light for photosynthesis in *Chlorella vulgaris* cells, which can hinder the growth and biomass production (Morales et al., 2018).

3.2.3 Reduction of nutrients concentration during cultivation time

Adequate nutrient supply promotes algal growth and enhances biomass productivity. It is observed that an external nutrient concentration, or the nutrient concentration in the culture medium, controls the microalgae growth phase. Tables 2 to 4 showed the pattern of orthophosphate and nitrate removal by microalgae during its growth cycle. Experiment 1, 2 and 3 showed the% removal of orthophosphate from the water by microalgae was 50%, 41.74% and 60.78% respectively, while the nitrate removal was 22%, 48% and 58% respectively during the growth cycle of 10 days. In the current study, this variability in the percentage of removed phosphorus may result from variations in the initial amount of phosphorus in the culture media and the cultivation conditions (Barghbani et al., 2012). The overall nitrogen content in the media gradually drops during the course of the growth process for all enriched CO_2 feed concentrations. This might result from Chlorella's vulgaris rapid anabolism in the initial few days of the cultivation phase (Barghbani et al., 2012).

Table 3. Experiment # 2 (ratios used 80 % water and 20 % culture with Intermittent 200 g CO_2).

	Duration of cultivation				
Parameters	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 10**
Temperature (C)	23.8	22.5	22.9	22.3	24
Solar irradiation (W/m^2)	412.6	377.8	410.9	390.4	367.8
pH	7.8	8	7.5	7.1	7.37
EC (μs/cm)	1.13	1.06	0.97	0.91	0.82
TDS (mg/L)	690	670	60	620	570
TSS (mg/L)	74	78	89	93	105
Orthophosphate (mg/L)	103	77	70	59	60
Nitrate (mg/L)	10	9.3	8.5	7.4	5.2
Absorbance (abs)	0.091	0.11	0.135	0.21	0.24
Biomass productivity (mg/L)	115.3	142.4	163.1	183.5	203.43

Table 4. Experiment # 3 (ratios used 80 % water and 20 % culture with Intermittent 300 g CO_2).

	Duration of cultivation				
Parameters	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 10**
Temperature (C)	24.1	24.3	23.9	25.3	24
Solar irradiation (W/m^2)	426.5	428.6	398.3	463.9	434.7
pH	8.5	7.7	7.5	7.6	7.1
EC (μs/cm)	1.34	1.27	1.35	0.99	0.95
TDS (mg/L)	740	720	690	60	580
TSS (mg/L)	81	87	102	99	110
Orthophosphate (mg/L)	102	89	85	65	40
Nitrate (mg/L)	10	8.1	7.3	5.8	4.2
Absorbance (abs)	0.12	0.25	0.27	0.38	0.49
Biomass productivity (mg/L)	120.3	211.5	225.6	302.7	318.76

The *Chlorella vulgaris* has consumed the maximum quantity of orthophosphates, which is an essential nutrient for microalgae, and it is a crucial component of nucleic acids, ATP (adenosine triphosphate), and phospholipids, playing a vital role in various cellular processes. Adequate phosphorus availability supports the growth, metabolism, and reproduction of *Chlorella vulgaris* cells (Sriwiriyarat and Mukhthong, 2021). The results highlight the dynamic interaction between nutrient availability and microalgae intake consumption, with substantial reduction in orthophosphate and nitrate concentrations that underscore the successful utilization of these nutrients by the microalgae, demonstrating a positive nutrient supply that promotes their growth and biomass productivity (Tavares et al., 2023).

3.2.4 Biomass productivity rate

Biomass productivity results presented in Tables 2, 3, and 4 showed that the biomass productivity in the initial two experiments # 1 and 2 were having gradual increasing; however, it was observed that experiment # 3 with 300 g of intermittent CO_2 was having drastically increasing pattern with respect to cultivation period. The biomass increasing pattern showed from first day of cultivation 113.3, 115.3 to 10th day of cultivation 120.3 to 196.6, 203.4 and 318.7 mg/L respectively.

Though the biomass productivity was not too much, it can be improved by optimizing the microalgae culture's exposure to sunlight and increasing the supply of carbon dioxide, potentially from various exhaust emission sources. This strategic approach aligns with the research's acknowledgment

that photoautotrophic species require substantial amounts of carbon to achieve optimal biomass output (Razzak, 2019).

Tabulated results from Experiment # 1 shows that biomass productivity increases over the cultivation time and maximum biomass concentration was found to be 197.5 mg/L, which is significantly lower than the findings of 2nd and 3rd experiments using CO_2 injections. Trial conducted at 200g CO_2 showed a progressive increase in biomass concentration during the cultivation phase.

The microalgae cultivated with ambient air and atmospheric CO_2 enrichment (referred to as atmospheric 0.03% CO_2), showed that the biomass concentration was slightly increased. Additionally, with 300 g of CO_2, the maximum concentration of biomass was obtained, this might be the result of the microalgae cultivated with higher concentration of CO_2 i.e., 300 g (Faruque et al., 2021).

3.3 Statistical analysis on biomass productivity

Tukey's Honest Significant Difference (HSD) test was used in the statistical analysis to determine the significance of differences between pairs of biomass productivity means at day 10 of cultivation period by using Minitab software (version 17) at a significance level of (α=0.05). The statistical analysis was conducted between control sample (experiment # 1) without CO_2 supplied from external source ($\times$1), experiment # 2 with 200 g of CO_2 supplied ($\times$2) and experiment # 3 with 300 g CO_2 supplied ($\times$3) into the photobioreactor cultivation system.

Table 5 showed that the p-value (6.306×10^{-14}) are highly significant and similar with difference between $\times$1 and $\times$3, followed by $\times$2 and $\times$3 as compared to control sample (experiment # 1) i.e., *p=0.0001*. Thus, it can be concluded that supplying CO_2 from external source in experiment # 2 and experiment # 3 have same pattern of statistical significance with co-relationship between two groups of means, having highly significant value with $\times$2 and $\times$3.

Table 5. Tukey HSD test comparative statement of difference of biomass productivity means.

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
$\times$1-$\times$2	6.8	0.4679	14.5344	4.7699	8.8301	2.0301	0.0001221
$\times$1-$\times$3	122.5	0.4679	261.833	120.4699	124.5301	2.0301	6.306×10^{-14}
$\times$2-$\times$3	115.7	0.4679	247.2986	113.6699	117.7301	2.0301	6.306×10^{-14}

3.4 Protein and lipid extraction yields

The microalgal biomass sample for protein and lipid extraction was collected, exhibiting the highest biomass productivity. The dried microalgal biomass protein content of 7.98% by weight was obtained using Lowrys method, and lipid yield of 1.87 g/ 5 g of dry biomass was obtained using n-hexane in solvent extraction method. The protein result indicates a relatively lower protein content compared to other studies conducted in the past. This divergence in protein content could be attributed to the experimental parameters employed in this study using ambient air. Unlike most research focused on wastewater; the approach involved utilizing exhaust gases from atmosphere contain substantial quantities of nitrogen oxides. Microalgae's biological elimination of nitrogen oxides is a potential method for converting nitrogen oxides into protein (Lam and Lee, 2012).

In the present case, the atmospheric nitrogen was basically introduced into the photobioreactor with low concentration, which is a macronutrient for microalgae growth and thus lower production of protein was observed. Studies utilizing synthetic medium with the green microalga *Scenedesmus dimorphus* in BG-11 under both indoor and outdoor cultivation settings have been observed in earlier research that *S. dimorphus* may produce cell biomass in outdoor environments with up to 35% protein and 37% total lipid under specific growing conditions. It was successfully demonstrated that the highest yields for protein and carbohydrates were

0.2 and 0.7 g/ L/ day, respectively, and could be obtained in the early stages of cultivation. The highest yield for lipids, 0.17 g /L/day, occurred in a late stage of cultivation. These results were obtained through a combination of manipulating nitrogen availability, light intensity, and cell inoculation density (Çoban et al., 2021). The amount of nitrogen in a culture medium determines the microalgae's cell development rate and biochemical composition. Research showed that nitrogen starvation in a culture medium slows down the cell growth rate of microalgae and reduces protein synthesis by increasing lipid or carbohydrate content (Razzak, 2019).

Table 6. Major fatty acids identified in the extracted lipids.

Name of fatty acid	g/100g Total fatty acids
Myristic (C14:0)	11.39
Palmitic (C16:0)	34.03
Palmitoleic (C16:1)	9.16
Stearic (C18:0)	5.38
Oleic (C18:1n9c)	16.58
Linoleic (C18:2n6c)	4.52
g-linolenic (C18:3n6)	13.23
Euric (C22:1n9)	5.66

3.5 Fatty acids compositional analysis

The microalgae lipids were extracted, and fatty acids compositional analysis was conducted presented in Table 6. The lipid composition was identified to include myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:1), and linolenic acid (C18:3). This detailed fatty acid profile provides information about the influence on biodiesel fuel properties. It is observed that all the major fatty acids were present in the extracted lipid, having favorable properties to produce microalgal biodiesel.

The literature emphasizes that the presence of long-chain saturated and monounsaturated fatty acids, particularly C18:2 and C18:3, significantly influences biodiesel cetane number, oxidation stability, and iodine content. It is commonly known that the qualities of biodiesel are closely related to the composition of free fatty acids. For example, oleic acid (C18:1) improves cold flow properties, whereas palmitic acid (C16:0) contributes to a lower iodine concentration and a larger cetane number. Additionally, the presence of linoleic acid (C18:2) and linolenic acid (C18:3) is associated with improved combustion properties, contributing to overall better fuel quality. The iodine value, a critical parameter for assessing biodiesel's chemical stability and susceptibility to oxidative rancidity, is significantly influenced by the presence of double-bonded fatty acids. A higher number of double bonds increases the polymerization potential, consequently reducing oxidation stability. This nuanced understanding of the fatty acid composition provides valuable insights into the potential applications and qualities of the biodiesel derived from the microalgae culture (Qie et al., 2019).

3.6 CO_2 injection into photobioreactor system

The intermittent CO_2 injected into the photobioreactor system with a velocity of 25 m/s of CO_2 for Experiment # 2 and Experiment # 3 represented 15 sec and 25 sec respectively. The area of the supply hose pipe was calculated as 2.54×10^{-4} m^2 and CO_2 velocity 25m/s, and the flow rate found was 6.36 $\times$ 10^{-3} m^3/s (using $Q = AV$). Now, this flow rate (Q) is multiplied by dosing time (t) using equation $V = Q \times t$, i.e., 15 sec and 25 sec to get the volume of CO_2 gas introduced into the photobioreactor cultivation system. The mass of CO_2 gas introduced into the photobioreactor tank were 200 g and 300 g, computed by ($D = M/V$) for Experiment # 2 and Experiment # 3 respectively.

3.7 Carbon dioxide (CO_2) bio-fixation by microalgae cultivation

The CO_2 bio-fixation rates (R_{CO_2}) are calculated using Equation 5 for all the three experiments conducted at alternative days. Therefore, in order to calculate the R_{CO_2}, the biomass productivity per day was calculated using the following regression equation obtained from a standard curve:

$$y = 0{,}7015x + 0{,}0362 \quad \left(R^2 = 0{,}9987\right) \tag{9}$$

The% C was assumed as 50% by dry weight of algal biomass as per literature (Scheufele et al., 2019) molecular weight of CO_2 44.01 g/mol and molecular weight of carbon is 12.01 g/mol.

Figure 3 showed that Experiment # 1 has a linear increasing trend in bio-fixation rate during the cultivation days with highest level to 362.07 mg/L/day.

Similarly, experiment # 2 also showed more or less a similar pattern but after 7th day of cultivation it increased to highest level to 374.92mg/L/day until 9th day of cultivation time. This increased level was due to introduction of intermittent CO_2 (200mg) into the photobioreactor for microalgae cultivation. Experiment # 3 showed a drastic increasing trend from 1st to 9th day of cultivation with introduction of 300mg of mass of CO_2 into the photobioreactor system. The lowest and highest level of bio-fixation rate were found to be 220.55 and 586.48 mg/L/day with 1st day and 9th day respectively.

High concentrations of carbon dioxide (CO_2) are essential for photosynthesis and have a direct impact on the growth rates at which microalgae is cultivated. The availability of CO_2 significantly impacts the efficiency of photosynthesis, thereby affecting biomass productivity. As a critical component of photosynthetic reactions, increased levels of CO_2 can enhance the efficiency of this process, ultimately leading to higher rates of biomass production (Wang et al., 2013). In the current study, it was found that introducing less CO_2 for a shorter amount of time causes it to dissolve completely due to the gas-liquid absorption process, whereas introducing more CO_2 for a longer amount of time reduces its ability to dissolve completely because the gas molecules cannot enter into the water molecules, reaching the equilibrium state (Razzak et al., 2024).

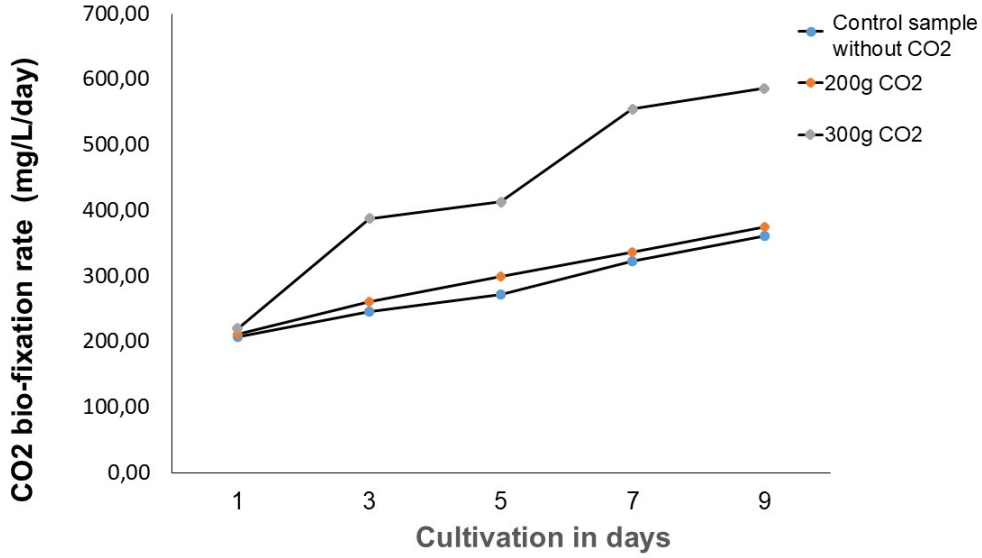

Figure 3. Microalgae CO_2 bio-fixation rate versus cultivation time.

3.8 Kinetic modelling of CO_2 volumetric mass transfer capacity

Multiplying the mass transfer coefficient of oxygen (k) by the surface area of the vessel in a photobioreactor provides an assessment of the system's capacity for both oxygen and carbon dioxide transfer. This combined product reflects the rate at which these gases can efficiently move into the culture medium within the photobioreactor, crucial for supporting the metabolic needs of the algae being cultured. The surface area of a rectangular photobioreactor with dimensions length (47.63 inch), width (12.6 inch) and height (35.43 inch) is calculated as 2734.08 $inch^2$ (1.763 m^2).

3.8.1 Volumetric Mass Transfer Capacity (k) for Oxygen:

Diffusion coefficient of oxygen in water (D_{O_2}) was 2×10^{-9} m^2 /s and thickness of the boundary layer (δ) is 0.02 meters.

$$k_{O_2} = \frac{2 \times 10^{-9} \times 1{,}763}{0{,}02} = 1{,}763 \times 10^{-7} \ m^3/s \quad (10)$$

3.8.2 Volumetric Mass Transfer Capacity (k) for Carbon Dioxide:

Diffusion coefficient of carbon dioxide in water (D_{CO_2}) is 1.9×10^{-9} m^2 /s and thickness of the boundary layer (δ) is 0.02 m.

$$k_{CO_2} = \frac{1{,}9 \times 10^{-9} \times 1{,}763}{0{,}02} = 1{,}676 \times 10^{-7} \ m^3/s \quad (11)$$

The rates at which oxygen and carbon dioxide molecules can move from the gas phase to the liquid phase of the gas-liquid interface are represented by the computed volumetric mass transfer capacity. A greater value denotes a faster gas transfer rate and higher quantity of gas transfer, which is necessary to sustain the algal metabolism, including photosynthesis.

4 Conclusion

This research study included the design and fabrication of an urban photobioreactor operated by solar energy system to cultivate microalgae species of *Chlorella vulgaris* for nutrient removal, biomass production, protein production and CO_2 sequestration. The research included monitoring of cultivation parameters for 10 days cultivation period. Three experimental conditions were compared, and it was found that experiment # 3 was having higher biomass productivity of 318.76 mg/L when introducing 300 g of intermittent CO_2 into cultivation system and nutrients removal efficiencies were 60.78% (orthophosphate) and 58% (nitrate). Statistical analysis found that introducing CO_2 from an external source in experiments # 2 and # 3 resulted in the same pattern of statistical significance, with a co-relationship between two sets of means (p value = 6.306×10^{-14}). The protein and lipid content yields were 7.98% and 37.4% by weight respectively. The O_2 and CO_2 volumetric mass transfer capacities for KO_2 and KCO_2 were 1.763×10^{-7} m^3/s and 1.676×10^{-7} m^3/s respectively.

The average O_2 and CO_2 transfer capability is improved by continuous agitation in a photobioreactor, essential for maintaining optimal conditions for the cultivation of microalgae. The extracted lipids contained favorable qualities of fatty acids for the production of microalgae biodiesel, myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:1), and linolenic acids (C18:3). An urban microalgae photobioreactors is an environmentally friendly strategy that can greatly advance the biobased economy and lessen the damaging impacts of CO_2 produced by conventional fossil fuel combustion on the environment.

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Conflict of interest

The authors declare that they have no relevant conflicts of interest. All co-authors have examined the work and agree with its contents. They have no financial interests to report. Furthermore, the writers affirm that the submission is their original work and is not being reviewed for publication.

Author's contribution

S.K.: Research, methodology and writing original draft. M.A.: Conceptualization, supervision, funding acquisition, formal analysis and editing. A.M.: Writing -reviewing and editing. A.I.: Writing and data processing.

References

Ali, M., Masood, A., and Saleem, M. (2021). Microalgae cultivation in wastewater for simultaneous nutrients removal and biomass production. *International Journal of Energy and Environmental Engineering*, 12:475–485. Online:https://n9.cl/answax.

Amaral, A., Gillot, S., Garrido-Baserba, M., Filali, A., Karpinska, A., Plósz, B., De Groot, C., Bellandi, G., Nopens, I., and Takács, I. (2019). Modelling gas-liquid mass transfer in wastewater treatment: when current knowledge needs to encounter engineering practice and vice versa. *Water science and technology*, 80(4):607–619. Online:https://n9.cl/5vgwj.

APHA (2005). *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, 21st edition edition.

Barghbani, R., Rezaei, K., and Javanshir, A. (2012). Investigating the effects of several parameters on the growth of chlorella vulgaris using taguchi's experimental approach. *International Journal of Biotechnology for Wellness Industries*, 1(2):128. Online:https://n9.cl/iohjx.

Borowitzka, M. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of biotechnology*, 70(1-3):313–321. Online:https://n9.cl/shstm.

Brindhadevi, K., Mathimani, T., Rene, E., Shanmugam, S., Chi, N., and Pugazhendhi, A. (2021). Impact of cultivation conditions on the biomass and lipid in microalgae with an emphasis on biodiesel. *Fuel*, 284:119058. Online:https://n9.cl/9isnj.

Çoban, A., Şimşek, G., and Çetin, A. (2021). Effect of nitrogen source on growth and protein and lipid amounts of a freshwater microalga scenedesmus acutus. *Turkish Journal of Science and Technology*, 16(2):215–220. Online:https://n9.cl/m9igzn.

Faruque, M., Mohammed, K., Hossain, M., and Razzak, S. (2021). Influence of elevated co2 concentrations on growth, nutrient removal, and co2 biofixation using chlorella kessleri cultivation. *International Journal of Environmental Science and Technology*, 18:913–926. Online:https://n9.cl/qhk2pc.

Gaurav, K., Neeti, K., and Singh, R. (2024). Microalgae-based biodiesel production and its challenges and future opportunities: A review. *Green Technologies and Sustainability*, 2(1):100060. Online:https://n9.cl/znc4x.

Lam, M. and Lee, K. (2012). Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnology advances*, 30(3):673–690. Online:https://n9.cl/w9fok.

Leflay, H., Pandhal, J., and Brown, S. (2021). Direct measurements of co2 capture are essential to assess the technical and economic potential of algalccus. *Journal of CO2 Utilization*, 52:101657. Online:https://n9.cl/ng2a3.

Mahapatra, S., Kumar, D., Singh, B., and Sachan, P. (2021). Biofuels and their sources of production: A review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. *Energy Nexus*, 4:100036. Online:https://n9.cl/8wa5y.

Morales, M., Sánchez, L., and Revah, S. (2018). The impact of environmental factors on carbon dioxide fixation by microalgae. *FEMS microbiology letters*, 365(3):fnx262. Online:https://n9.cl/h6rnz.

Nezammahalleh, H., Ghanati, F., Adams, T., Nosrati, M., and Shojaosadati, S. (2016). Effect of moderate static electric field on the growth and metabolism of chlorella vulgaris. *Bioresource technology*, 218:700–711. Online:https://n9.cl/5rotd.

Qie, F., Zhu, J., Rong, J., and Zong, B. (2019). Biological removal of nitrogen oxides by microalgae, a promising strategy from nitrogen oxides to protein production. *Bioresource technology*, 292:122037. Online:https://n9.cl/fmenw.

Razzak, S. (2019). In situ biological co2 fixation and wastewater nutrient removal with neochloris oleoabundans in batch photobioreactor. *Bioprocess and biosystems engineering*, 42(1):93–105. Online:https://n9.cl/yq31m7.

Razzak, S., Bahar, K., Islam, K., Haniffa, A., Faruque, M., Hossain, S., and Hossain, M. (2024). Microalgae cultivation in photobioreactors: Sustainable solutions for a greener future. *Green Chemical Engineering*, 5(4):418–439. Online:https://n9.cl/2lhn0.

Rehman, M., Kesharvani, S., Dwivedi, G., and Suneja, K. (2022). Impact of cultivation conditions on microalgae biomass productivity and lipid content. *Materials Today: Proceedings*, 56:282–290. Online:https://n9.cl/nl31us.

Rodionova, M., Poudyal, R., Tiwari, I., Voloshin, R., Zharmukhamedov, S., Nam, H., Zayadan, B., Bruce, B., Hou, H., and Allakhverdiev, S. (2017). Biofuel production: challenges and opportunities. *International Journal of Hydrogen Energy*, 42(12):8450–8461. Online:https://n9.cl/0xwr4.

Saratale, R., Ponnusamy, V., Jeyakumar, R., Sirohi, R., Piechota, G., Shobana, S., Dharmaraja, J., Lay, C.-H., Saratale, G., and Shin, H. (2022). Microalgae cultivation strategies using cost–effective nutrient sources: Recent updates and progress towards biofuel production. *Bioresource Technology*, 361:127691. Online:https://n9.cl/srjve.

Scheufele, F., Hinterholz, C., Zaharieva, M., Najdenski, H., Módenes, A., Trigueros, D., Borba, C., Espinoza-Quiñones, F., and Kroumov, A. (2019). Complex mathematical analysis of photobioreactor system. *Engineering in life sciences*, 19(12):844–859. Online:https://n9.cl/xtk4h.

Shuler, M. and Kargi, F. (2002). *Bioprocess Engineering- Basic Concepts*. Prentice-Hall, second edition edition.

Sievert, K., Cameron, L., and Carter, A. (2023). Why the cost of carbon capture and storage remains persistently high. Online:https://n9.cl/grhfk.

Sriwiriyarat, T. and Mukhthong, M. (2021). The feasibility study of using microalgae for polishing consumer-products industrial effluent containing high total dissolved solids. *Thai Environmental Engineering Journal*, 35(1):1–14. Online:https://n9.cl/9kjzb.

Stojiljković, D. and Spasojević, I. (2023). Urban photo-bioreactor liquid3. Green Product Award. Online:https://n9.cl/xx5ch.

Tavares, L., Nudi, M., Arroyo, P., Godoy, R., and Trevisan, E. (2023). Effect of different concentrations of phosphorus and nitrogen on the growth of the microalgae chlorella vulgaris. *International Journal of Energy and Environmental Engineering*, 14(4):563–572. Online:https://n9.cl/vmaxgx.

United Nations (2024). Causes and effects of climate change. United Nations. Online:https://n9.cl/nij3r.

Valavanidis, A. (2023). Extreme weather events exacerbated by the global impact of climate change. glimpse of the future, if climate change continues unabated. Online:https://n9.cl/5nffq.

Wang, L., Li, Y., Sommerfeld, M., and Hu, Q. (2013). A flexible culture process for production of the green microalga scenedesmus dimorphus rich in protein, carbohydrate or lipid. *Bioresource Technology*, 129:289–295. Online:https://n9.cl/ghase.

Yen, H., Ho, S., Chen, C., and Chang, J. (2015). Co2, nox and sox removal from flue gas via microalgae cultivation: A critical review. *Biotechnology journal*, 10(6):829–839. Online:https://n9.cl/dy725.