



## Original Research Paper

# Isolation and Morphological identification of fast-Growing Microalgal Species with High Biomass Productivity

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### Abstract

Microalgae have been recognised as a valuable renewable source of bioenergy because of their rapid growth rates, high photosynthetic efficiency, and high capacity for biomass production. This work is focused on the identification and isolation of rapidly growing microalgal species with high biomass productivity from natural water bodies. Samples of algae were taken from different locations and processed using microbiological techniques that allow for enrichment, isolation, and purification. Pure cultures of microalgae were obtained in laboratory settings using BG-11 medium. Identification of isolated species was done based on morphological characteristics with the help of a light microscope and scanning electron microscope. The assessment of growth kinetics comprised the measurement of optical density at 750 nm (OD<sub>750</sub>), while the biomass determination method was based on the technique of dry cell weight (DCW). The specific growth rate ( $\mu$ ) and biomass productivity were quantified to assess variations in growth performance among the isolates. The results of the study demonstrated that this work has successfully identified the microalgal strains that showed the highest growth rates and yielded the greatest biomass. The strains reveal great potential for bioenergy production, bioproduct synthesis, and sustainable biotechnological practices.

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## 1. Introduction

The isolation and identification of species of microalgae is an important step in strain selection studies. Isolation techniques help to collect single microalgal cultures from mixed communities in nature, keep them in a relatively pure state and maintain them in laboratory conditions (Ishfaq et al.,2026). After isolation, the first step is identification of microalgae is morphological characterization. Morphological traits include the shape of the cell, size, arrangement, presence of structures, formation of colonies, pigmentation and organization of cells (Báez et al.,2025). Use of a microscope with proper illumination and magnification gives important information for preliminary identification by its morphology (Wortz et al.,2026). Due to its simplicity and cost effectiveness, morphological identification is very important as a preliminary method before detailed identification of species performed by molecular methods (Marinova et al.,2025) Conducting a comparative analysis of various parameters such as optical density, dry weight of algal cells, and biomass productivity facilitates the identification of fast-growing species with excellent biomass productivity that could be further optimized and utilized for the production of microalgal biomass

and biological carbon dioxide capture (S, Vinayaka et al.,2026). The identification of strains that can grow quickly and produce a lot of biomass is among the most significant requirements for achieving sustainable microalgal biotechnology (Xuejie et al.,2026). Wild microalgae that are found in different aquatic habitats are known to have several characteristics that help them grow much faster under diverse conditions (Ambily et al.,2024). Therefore, the need for isolation and screening of natural microalgae species is paramount to selecting the most useful strains that can be used in industrial production activities and thus contribute to environmental management, energy production, reduction of greenhouse gases, and the achievement of various sustainable development goals (Melendez et al.,2025). Figure 1 depicts how systematic isolation and screening of indigenous microalgae contribute towards the identification of efficient and effective strains for environmental and industrial applications (Amin et al.,2025).

This research strives to isolate, identify, and study the rapidly growing microalgae that can produce high biomass utilising classical microbiological methods and practical approaches in physiology.

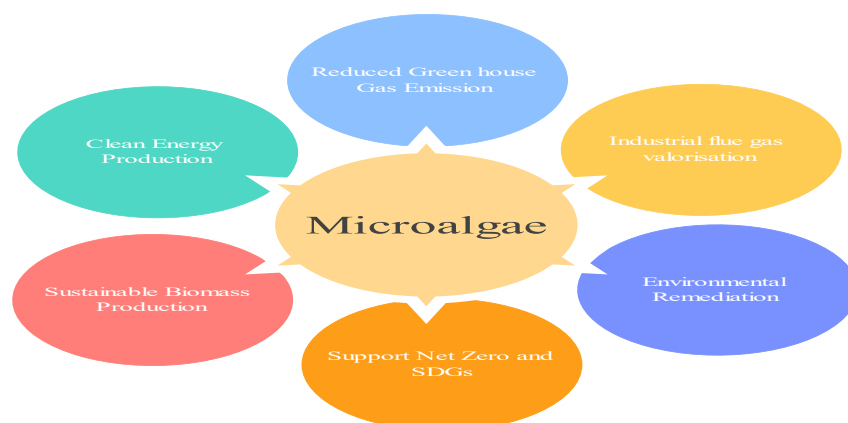


**Figure 1. Environmental and Sustainable Development Benefits of Microalgae.**

## 2. Materials And Methodology

The objective of the research was to identify and assess native microalgae strains that show promising results in terms of their growth rate and biomass production. Environmental samples were taken from certain freshwater sites, and enrichment was performed under laboratory conditions in order to achieve the required growth of microalgae species. Different microalgal strains were isolated by means of dilution, plate outcomes, and serial subculturing procedures, and were purified afterwards (Al-Saeedi et al.,2026). The purified samples were morphologically characterized and

cultivated in controlled conditions. Their growth was recorded at regular time intervals by means of the optical density method and biomass recording. Various indicators have been evaluated and characterized, including growth rate, biomass concentration, and biomass productivity. The conducted analysis enabled the selection of the most promising microalgae strains for future research. The experimental workflow from sample collection to Biomass productivity analysis led to the selection of fast-growing microalgal species with high biomass productivity, as shown in Figure 2.

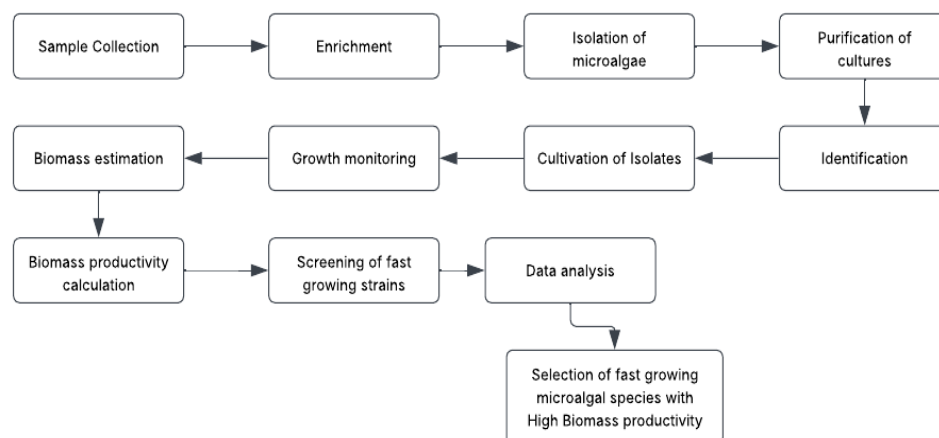


**Figure 2. Experimental Workflow for High-Biomass Microalgal Selection**

### 2.1 Sample Collection

The research involved Mahendergarh district, Haryana, India, as the sampling point from where microscopic algae were collected from four designated localities, Pali, Rewasa, Sisoth, and Dholi appear in Figure 3. The sites were chosen so that they can represent various freshwater ecosystems of Mahendergarh district. By analyzing the sampling map, it can be evidently seen that the district of Mahendergarh is situated in the

southwestern region of Haryana. The map represents the boundary of the district within the state also depicts the geographical location of Mahendergarh district with the help of inset maps. The map also depicts district boundaries, major rivers/streams, water bodies, and the precise sampling locations marked with red dots. The selected sites are distributed across different regions of the district, ensuring representative sampling for the isolation and morphological identification of fast-growing microalgal species with high biomass productivity.

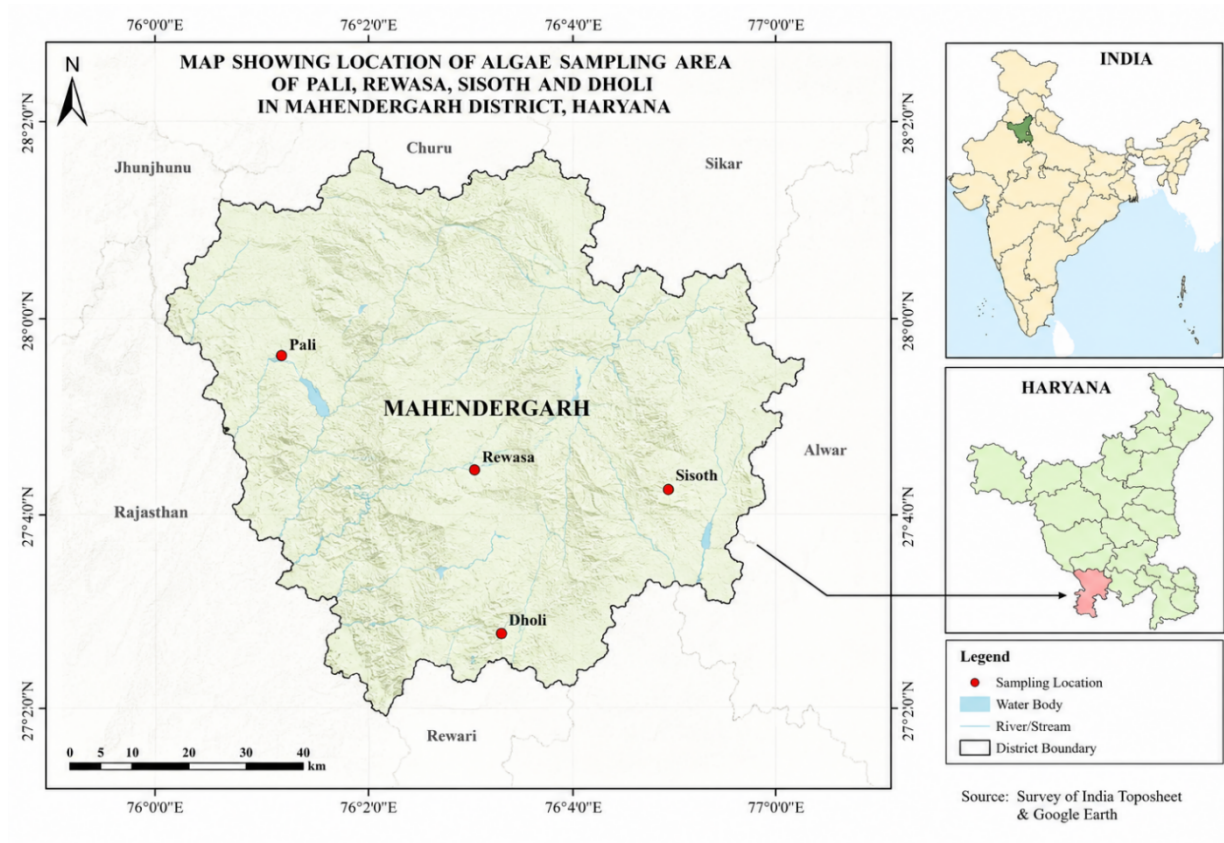


**Figure 3. Location Map of Microalgal Sampling Sites in Mahendergarh District, Haryana, India.**

Water samples containing microalgal species were collected from various freshwater environments, including ponds, lakes, rivers, and wastewater sites by using sterile sampling bottles, and after labelling, samples were transported to the

laboratory under refrigerated conditions (approximately 4°C) for further processing shown in Figure 4.



**Figure 4. Sampling Sites and sample pictures in Mahendergarh District, Haryana, India.**

## 2.2 Culturing and culture media

Each water sample (10–20 mL) was inoculated into 100 mL of sterile BG-11 medium to enrich microalgal growth. The cultures were incubated at  $25 \pm 2^\circ\text{C}$  under a 16:8 h light photoperiod with a light intensity of 2000–3000 lux for 24–48 h. BG-11 media was prepared by dissolving the required macronutrients ( $\text{NaNO}_3$ -1.5g,  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ - 0.04 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.075 g,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ - 0.036 g,  $\text{Na}_2\text{CO}_3$ - 0.02 g) organic acid/chelators (Citric acid- 0.006 g, Ferric ammonium citrate- 0.006 g,  $\text{Na}_2\text{EDTA}$ - 0.001 g) and  $1 \text{ mL L}^{-1}$  of trace metal solution ( $\text{H}_3\text{BO}_3$ - 2.86 g/L,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ -1.81 g/L,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.222 g/L,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ - 0.39 g/L,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -0.079 g/L,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ - 0.049 g/L) in distilled water. The pH was adjusted to 7.1–7.5, followed by sterilisation at  $121^\circ\text{C}$  for 15–20 min in an autoclave. After cooling to room temperature, the medium was stored at  $4^\circ\text{C}$  in the dark until use.

## 2.3 Isolation of Microalgal Strains

Microalgal strains were isolated using various techniques, such as serial dilution or streaking plate methods. The cultures were diluted several times before spreading them onto BG-11 plates for culturing and isolating well-separated colonies using the streaking plate method (Gropali et al., 2026). Furthermore, individual cells or colonies were inoculated into fresh BG-11 medium using a sterile micropipette with microscopic monitoring. The cultures were then incubated under laboratory conditions until

visible colonies appeared. Pure cultures were obtained by continually sub-culturing them in fresh BG-11 agar plates. The purity of each isolate was confirmed by microscopy to check the absence of bacterial and fungal contamination before being maintained in liquid BG-11.

## 2.4 Morphological Identification

The microalgae isolates that passed through purification were analyzed morphologically. The analysis was done using a light microscope with  $40\times$ – $100\times$  magnification and involved the consideration of cell shape, size, colony formation, structure of chloroplast, characteristics of the cell wall, and motility based on standard taxonomic keys (Al-Saeedi et al., 2025). As a result of the morphological analysis, dominant isolates were identified as *Chlorella sorokiniana* and *Scenedesmus obliquus*. After that, the isolates were cultured in BG-11 medium under controlled laboratory conditions at  $25 \pm 2^\circ\text{C}$ , with a 16:8 h light photoperiod, 2000–4000 lux light intensity, and pH 6.8–7.5. Cultures were gently aerated or manually shaken to ensure uniform growth and proper nutrient distribution. During the preliminary screening, *C. sorokiniana* exhibited superior growth performance in BG-11 medium compared with the other tested microalgal species. The strain demonstrated a rapid growth rate and efficient biomass accumulation under the experimental conditions. Based on its higher growth potential and fast biomass production, *C. sorokiniana* was selected as the target microalgal species for subsequent experimental investigations.

## 2.5 Growth monitoring

Measurements of optical density (OD) were conducted at 750 nm for 14 days to keep track of the

development of microalgae. With the plotting of growth curve and the computation of specific growth rate ( $\mu$ ), the effectiveness at which different isolates grow could be observed. As for biomass measurement, the dry cell weight (DCW) procedure is used whereby the growth medium undergoes centrifugation, washing of the biomass with distilled water and drying of the biomass at a temperature of 60–80°C in order to attain a constant weight. Quantities of biomass produced are expressed in grams per liter ( $\text{g L}^{-1}$ ), while calculations of productivity of biomass based on the final value of dry biomass produced are expressed in terms of grams per litre per day ( $\text{g L}^{-1} \text{day}^{-1}$ ). Considering factors such as growth rate, optical density, dry cell weight, biomass productivity, and general growth trend, the strains were screened to prioritise them for further studies.

## 2.6 Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean  $\pm$  standard deviation. For statistical analysis, data were analyzed using one-way ANOVA to find out the differences between microalgal isolates. The final selection of microalgal strains was made based on their growth performance, which includes specific growth rate, dry biomass yield, biomass production and overall growth characteristics. The isolates that showed the highest biomass production were regarded as fast-growing isolates for further biotechnological application (Akyüz et al., 2025).

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and Identification of microalgae

Using techniques like serial dilution, streak plate method, and micropipette isolation, microalgal species originating from freshwater habitats in the Mahendergarh district were enriched and isolated. After several transfers on the BG-11 agar medium under optimal conditions in the lab, pure axenic cultures were obtained. This was confirmed by the microscopic analysis which showed no bacterial or fungal contamination.

Classified based on morphological features observed in light microscopy and using scanning electron microscopy, two predominant microalgal species were identified as *Chlorella Sorokiniana* and *Scenedesmus obliquus*. But since the isolates of *C. Sorokiniana* exhibited good growth on the BG-11 medium all throughout the cultivation time, this was selected for analyzing of growth rate and biomass yield as it showed rapid growth and consistently excellent growth characteristics throughout the study.

### 3.2 Morphological features of isolated microalgae

To examine the morphological features of *Chlorella sorokiniana*, it was subjected to Scanning Electron Microscopy (SEM) analysis. The SEM pictures of the *C. Sorokiniana* species are shown in Figure 5.

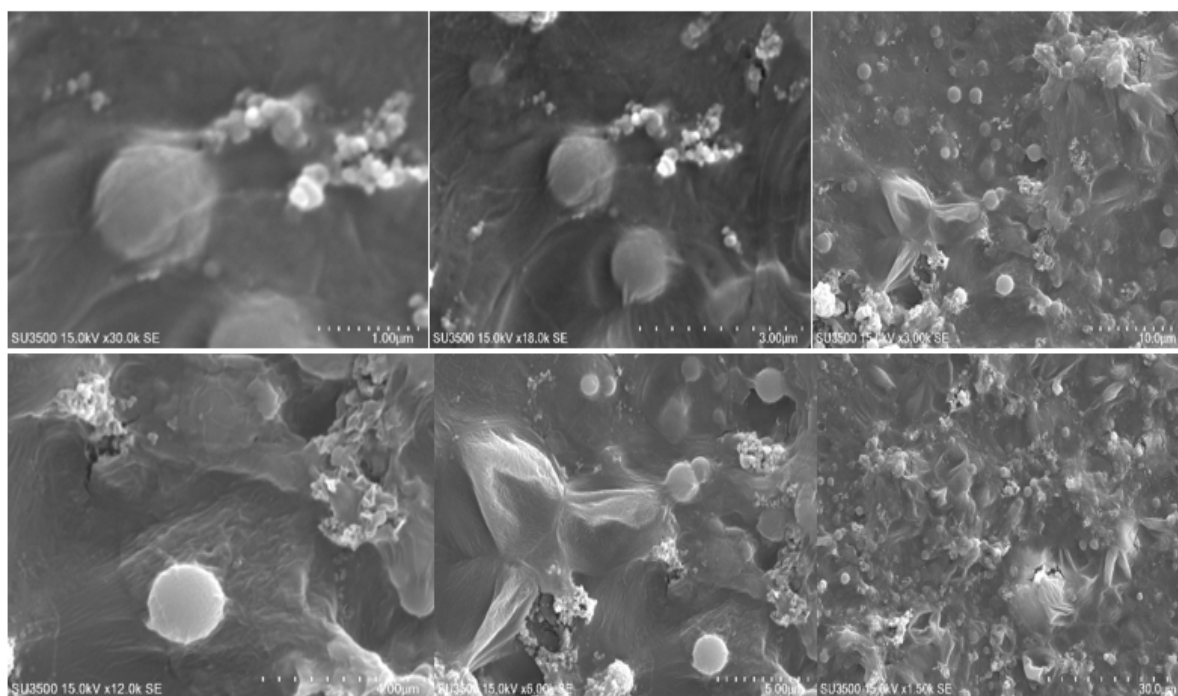


**Figure 5. SEM (Scanning Electron Microscope) images of *Chlorella sorokiniana* species**

The isolated microalgal species possessed clear morphological traits supporting their identification. *C. sorokiniana* species was represented by small, unicellular cells ranging from spherical to elliptical in shape with a size of roughly 3-8 µm. Cells were characterized by having a smooth cell wall and a single cup-like chloroplast filling most of the cell volume. The isolated cells were observed to exist individually with exotic green colouration attributed to high amounts of chlorophyll pigments. The recorded morphological characteristics were consistent with taxonomic descriptions provided in earlier reports, confirming the identification made. The pure and good morphological status of isolates suggested their suitability for further studies addressing productivity in terms of biomass. For daily observations, biomass productivity (P, g/L/day) is calculated as:  $P = \frac{X_i - X_{i-1}}{\Delta t}$  where:  $X_i$  = DCW at the current day (g/L),  $X_{i-1}$  =

DCW at the previous day (g/L),  $\Delta t$  = 1 day

### 3.3 The optical density (OD750)

The optical density (OD750) of *C. sorokiniana* increased continuously from the beginning of the 14-day cultivation. There was a sudden rise in optical density between Day 6 and Day 9. Following that phase, a gradual increase was noted, suggesting that the stationary phase had been approached (Fig 6A). An association was found between dry cell weight (DCW) and optical density of *C. sorokiniana*, which shows that OD increases with an increase in biomass concentration. Thus, it is evident that OD can be called a good indirect tool for measuring microalgal biomass production (Fig 6B). The dry cell weight of *C. sorokiniana* increased continuously throughout the experiment, showing that biomass was being grown continuously. The maximum weight of biomass was reached by the end of the experiment, indicating the effectiveness of the growth process (Fig 6C). The

biomass productivity of *C. sorokiniana* changed during the experiment, with its maximum value during the period of active growth of algae.

The fluctuations reflect changes in growth rate, while the overall trend indicates efficient biomass production by the selected microalgal isolate (Fig 6D).

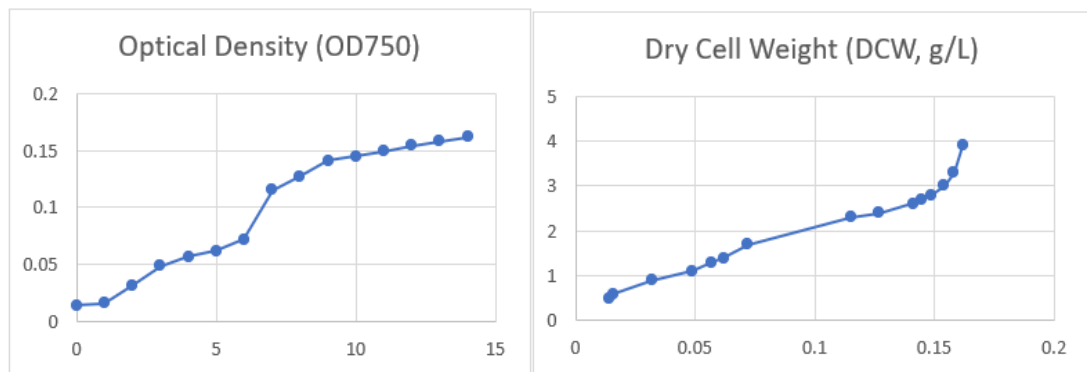


**Fig 6 (A) Graph showing the growth of *Chlorella sorokiniana* species during days. (B)Graph showing the growth of *Chlorella* species in relation to dry cell weight**

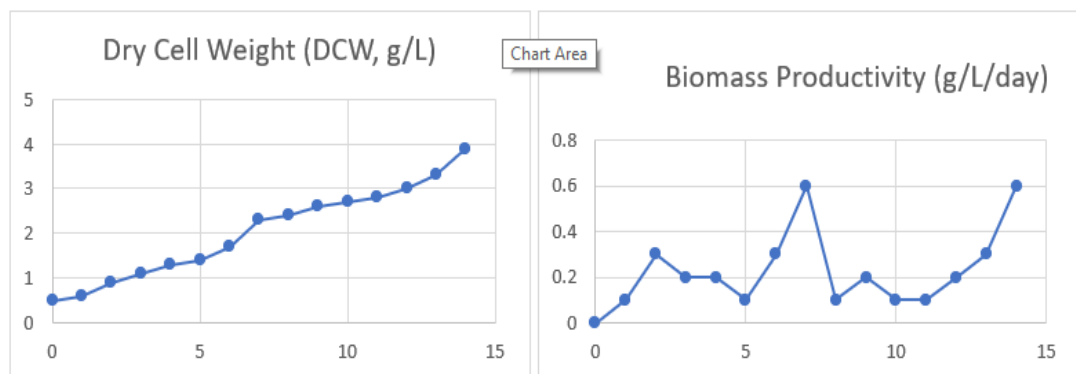


**Fig 6( c) Graph showing Dry Cell Weight During Cultivation, Fig 6(D) Graph showing Biomass Productivity Over Time**

## Conclusion

Morphological characterization through light microscopy and scanning electron microscopy facilitated the identification of two main microalgal species, namely *Chlorella sorokiniana* and *Scenedesmus obliquus*. Out of the isolated strains, *C. sorokiniana* exhibited excellent, stable, and fast growth rates under BG-11 cultivation conditions and thus was chosen for further investigations. The

morphological features of *C. sorokiniana*, including its small unicellular rounded to oval-shaped cells and specific chloroplast structure, coincided with the features used for its taxonomy.

The process of growth analysis revealed that *C. sorokiniana* continuously accumulated biomass during the 14-day growth period. The increase in optical density at OD<sub>750</sub> positively correlated with the increase of dry cell weight, which signifies the potential use of optical density as an approach to

measure biomass. The maximum biomass was reached by the end of the cultivation period and the maximum biomass production was achieved during the period of active growth. Overall, the results obtained show that local strains of microalgae, especially *Chlorella sorokiniana*, may be considered potential candidates for high-biomass cultivation. The systematic approach to the selection of microalgae high-efficiency strains includes their isolation, morphological identification, growth monitoring, and determination of biomass productivity and may serve as a reasonable approach for selection of high-performance strains of microalgae. The selected strain may be investigated for the optimization of cultivation conditions and CO<sub>2</sub> tolerance and flue gas exposure experiments. Thus, the present research study is a good basis for further future developments of microalgae-based technologies.

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