The influence of varying concentrations of CO_2 on the buildup of carbohydrates in microalgae biomass

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Abstract. Microalgae have gained popularity over the century due to their numerous intrinsic properties superior to higher plants, making them a potential target and feedstock for the development of biotechnological products in various fields. The storage of carbohydrates within microalgae cells positions them as a promising reservoir for biofuel production and a range of other valuable bioproducts, encompassing biological compounds, nutritional supplements, and more. Key determinants influencing microalgae carbohydrate levels comprise nutrient availability, light intensity, temperature, and CO_2 concentrations. In this context, the CO_2 concentration plays a key role, as it is one of the main factors influencing the photosynthetic processes. This study aimed to explore the impact of different CO_2 concentrations on the carbohydrate profile of biomass sourced from *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadicauda* B-1. The findings revealed that a CO_2 concentration of 2% v/v notably enhanced carbohydrate accumulation, reaching 75.5% for *Chlorella vulgaris* sp. BB-2 and 72.0% for *Scenedesmus quadicauda* B-1.

1 Introduction

The constant need to find new alternatives for the production of environmentally friendly products has led to the discovery and development of new technologies and biological models for the production of bioproducts in various fields such as food, cosmetics, healthcare, energy and others. The use of microalgae for the production of bioproducts has become highly relevant due to the continuous need for environmentally friendly and sustainable solutions in various industries. They have relatively high photosynthetic efficiency and growth rates, and some species grow well in brackish or seawater, as well as in wastewater of various origins. Microalgae are known for their potential to produce bioproducts and are promising targets for biotechnology due to their lipid, carbohydrate, protein content and photosynthetic activity [1, 2].

Carbohydrates are a diverse group of organic compounds, including mono-, oligo- and polysaccharides, and play a key role in microalgae. These organic compounds perform many functions in cells, such as providing cell wall structure, storing energy for metabolic processes and ensuring survival under low light conditions. They are also involved in the adaptation mechanisms of microalgae to changing environmental conditions, providing stability and the necessary resources for continued survival. The carbohydrate content of microalgae varies between species and depends on cultivation conditions [3, 4]. The accumulation of large amounts of carbohydrates in microalgae cells and their ability to utilize waste products, including wastewater and CO_2 flue gases, allows microalgae to be used as a resource for converting waste into valuable raw materials, including the production of biofuels such as butanol. The production of biobutanol requires carbohydrate-rich feedstocks. Microalgae capable of accumulating significant amounts of carbohydrates under natural environmental conditions are known, including genera such as *Chlorella, Spirulina, Scenedesmus, Chlamydomonas, Porphyridium, Spirogyra*, etc., which are actively used for research and practical applications in bioenergy, food and cosmetics, medicine and environmental biotechnology. However, there are barriers to scale-up processes for the production of biobutanol and other products that require even larger amounts of biomass with a rich carbohydrate composition [5]. One promising solution is to optimize microalgae cultivation by modifying abiotic cultivation conditions such as temperature, humidity, pH and nutrient composition. Carbon dioxide (CO_2) from various sources, including atmospheric CO_2 in the form of carbonates ($NaHCO_3$, Na_2CO_3) and CO_2 emissions from industrial processes, plays a role in the accumulation of carbohydrates in microalgae biomass [6]. The effect of CO_2 concentration on growth and carbohydrate accumulation in microalgae biomass is an important aspect of research. Despite existing studies, there are still many questions regarding the optimal cultivation conditions and the mechanisms regulating carbohydrate accumulation in different species and strains of microalgae.

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Therefore, this study focuses on optimizing the cultivation of productive strains of the microalgae *Chlorella vulgaris* and *Scenedesmus quadicauda* using different CO_2 concentrations to increase carbohydrate yield. This approach aims to find efficient methods to maximize the potential of microalgae for the production of bioproducts and to improve the resilience of industrial processes to environmental challenges.

2 Materials and Methods

The research employed pure cultures of the *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadicauda* B-1, shown in Figure 1, were taken from the collection of the Laboratory of Photobiotechnology of the Al-Farabi KazNU.

Cultivation of microalgae strains *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadicauda* B-1 were connected to a standard Tamiya composition medium for 12 days with fluorescent lamps at 4000 Lux and a temperature of 25-30^{0}C. The dry weight of the microalgae cell biomass was assessed following the method outlined in reference [7].

The effect of different carbon dioxide concentrations on dry biomass yield and carbohydrate buildup in microalgae was investigated. For this purpose, microalgae were grown under aeration conditions with an air mixture containing 0.5%, 1%, and 1.5% v/v CO_2 for 12 days. A Boyu air compressor with an S-4000B air pump was used for aeration, and a PMA-0.063 G rotameter was used to regulate the CO_2 concentration. Aerated air lacking CO_2 was utilized for control conditions.

The phenol-sulphuric acid method was used to analyze the total carbohydrate content (TCC). In this method, 0.16 ml of the microalgae sample was added to 0.16 ml of 5% phenol solution, after which 0.8 ml of concentrated sulphuric acid was added to the mixture. The resulting mixture was thoroughly mixed and heated in a water bath for 10-20 minutes. The light absorbance at a wavelength of 490 nm was then measured using a spectrophotometer. Aqueous glucose solution at different concentrations was used as a control sample to construct a standard absorbance curve. The determination was carried out in triplicate [8].

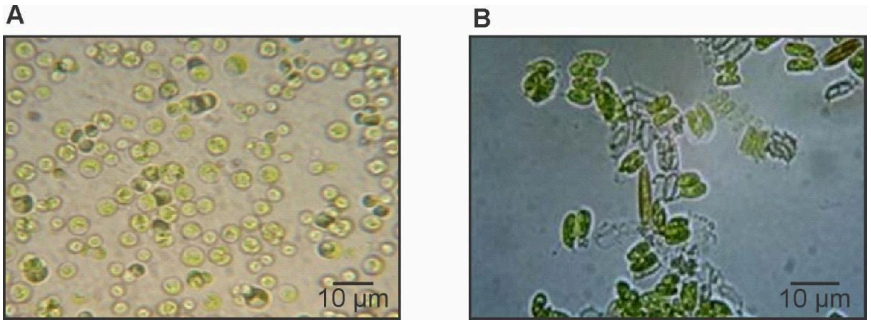

Fig. 1. Microphotographs of microalgae: A – *Chlorella vulgaris* sp. BB-2 and B – *Scenedesmus quadricauda* B-1.

3 Results and discussion

The impact of varying CO_2 concentrations on the growth of dry biomass from microalgae strains *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 was investigated. Figure 2 shows the results of the accumulation of dry biomass of microalgae in experiments with CO_2 concentrations of 1.5, 2, and 2.5% v/v. Dry biomass of *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 increased at 2% v/v CO_2 concentration, while at 1.5 and 2.5% CO_2 concentrations it was comparatively lower. After 12 days of cultivation, the maximum accumulation of dry biomass for the microalgae strains *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 were 3.3 g/L and 2.85 g/L at a concentration of 2% v/v, respectively. At CO_2 concentrations of 1.5% and 2.5% v/v, the dry biomass accumulation for *Chlorella vulgaris* sp. BB-2 was 2.75 g/L and 2.89 g/L, respectively. Similarly, for *Scenedesmus quadricauda* B-1, it was 2.53 g/L and 2.62 g/L, respectively. Thus, for microalgae strains *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1, the highest accumulation of dry biomass was achieved in the experiment with 2% v/v CO_2.

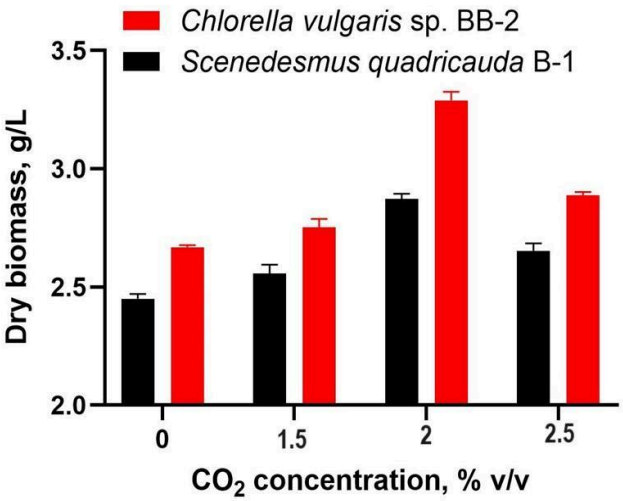

Fig. 2. The accumulation of dry biomass in *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 was observed at various CO_2 concentrations, including 0%, 1.5%, 2%, and 2.5%

After 12 days of cultivation, the total carbohydrate content (TCC) of the microalgae reached maximum values at 2% v/v CO_2, exceeding the values at 1.5% v/v CO_2 and the control (0% v/v CO_2). This indicates optimal biomass accumulation under conditions of increased CO_2 concentration. Figure 3 shows the TCC content at different CO_2 concentrations in the range of 74-75% dry cell weight for *Chlorella vulgaris* sp. BB-2 and 68-71% dry cell weight for *Scenedesmus quadricauda* B-1. The findings of the study affirm that CO_2 facilitates the vigorous growth of microalgae. Specifically, a CO_2 concentration of 2% v/v leads to the most significant enhancement in biomass for both *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1, increasing by 1.50 and 1.48 times, respectively.

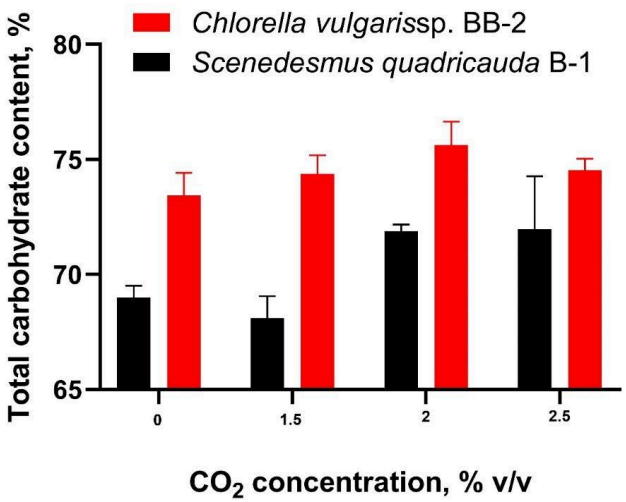

Fig. 3. Total Carbohydrate Content in the biomass of *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 varied across different CO_2 concentrations, specifically at 0%, 1.5%, 2%, and 2.5% v/v.

The results of subsequent studies have also shown that an increase in the carbon dioxide content of the influent gas enhances the process of carbohydrate accumulation in microalgae cells [9, 10, 11]. As an example, Chen and colleagues [12] observed fluctuations in microalgae carbohydrate content, ranging from 16% to 60%, correlating with CO_2 concentration. The buildup of carbohydrates in microalgae cells stems from CO_2 fixation

during the process of photosynthesis. Sufficient CO_2 provision stands out as a pivotal factor affecting carbohydrate buildup in microalgae cells. Several research indicate that augmenting the CO_2 percentage in the influent gas enhances carbohydrate buildup in microalgae. For example, increasing the concentration of dissolved CO_2 from 3 to 186 μmol/L when cultivating *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* can increase the carbohydrate content from 9.3% to 21% and from 3.19% to 7.4%, respectively [13]. Cheng and colleagues also investigated the effect of 2% CO_2 concentration on the proportional content of cell wall carbohydrates, starch and lipid in microalgae of the genus *Chlorella* (*C. vulgaris, C. sorokiniana, C. minutissima, C. variabilis*) [14]. Increased CO_2 concentration (2%) caused an increase in starch in two cultures (*C. variabilis, C. vulgaris*), an increase in lipids in only one culture (*C. minutissima*), and an increase in cell wall carbohydrate composition in three strains (*C. vulgaris, C. sorokiniana, C. variabilis*). The content of both starch and cell wall carbohydrates in *C. minutissima* microalgae after cultivation at high CO_2 concentration was not significantly different from the ambient CO_2 concentration. The results of this study highlight the importance of an individual approach to optimizing CO_2 concentration to maximize carbohydrate buildup in different species and strains of microalgae.

4 Conclusion

The results of the study support the promising potential of using microalgae in the biorefinery industry, supporting their use as a sustainable feedstock. The findings indicate that microalgae, particularly when exposed to elevated CO_2 concentrations, efficiently accumulate carbohydrates. This efficiency makes them an attractive option for producing biological products and third-generation biofuels. Specifically, the strains *Chlorella vulgaris* sp. BB-2 and *Scenedesmus quadricauda* B-1 show the highest dry biomass accumulation at a CO_2 concentration of 2% v/v. Among these, *Chlorella vulgaris* sp. BB-2 stands out for releasing greater TCC at increased CO_2 levels, especially at 2% v/v, which underscores its potential for biobutanol production. The development of an efficient, cost-effective, and feasible biorefinery that leverages microalgae for carbon dioxide capture and biomass utilization for biofuel and other product production is a promising direction. This approach not only requires close attention but also represents a significant step towards advancing environmentally sustainable energy efficiency technologies.

CRediT authorship contribution statement

MOB, BKD, MAL literature, experimental works, and writing; MOB, FKS, DEZ literature, plan survey, and analysis; MOB, main idea, consulting, writing. All the authors contributed to the writing of the final version and approved the manuscript.

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