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Dynamics of primary and secondary metabolites in *Chlorella vulgaris* exposed to biological stress and varied harvesting periods

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Abstract

Objective

Chlorella vulgaris is a microalga that is a promising candidate for solving many problems due to its ease of cultivation, rapid growth, absorption of carbon dioxide, and production of oxygen during its growth, as well as its production of many primary and secondary compound. This study aimed to investigate the effect of different concentrations of biocarbon, days variation of harvesting cells, and their interaction in Chlorella vulgaris. Also, it aimed to estimate the rate of protein, lipid, carbohydrate, and phenolic acid.

Materials and methods

In this study, the experiment was conducted in the plant tissue culture laboratory of Diyala University under sterile conditions. Algae were grown in BG11 medium at a temperature of about 25°C with a photoperiod of 16.8 hours. Biocarbon was added to the medium at concentrations of 300, 600, and 900 mg/L. Samples were collected on days 7, 14, and 21. Then, the amount of protein was measured by the Kjeldahl method, fat by the Soxhlet apparatus, carbohydrate by the phenol-sulfuric acid method, and flavonoid compounds by HPLC. The experiment was conducted in a completely randomized design with three replications, and the data were analyzed with SPSS software at a significance level of 0.05.

Results

It was observed that there was a direct correlation with increasing biocarbon concentration added to the culture medium; the highest values reached 61.666, 9.155, and 18.362 mg/L, respectively at a concentration of 900 ppm. Considering the time of cell harvesting, the highest values were found after 14 days from adding biocarbon to the culture medium. They reached 60.025, 8.540, and 17.741 mg/L for protein, lipid, and carbohydrate, respectively. According to the results of the phenolic acids, namely Rutin, Caffeic Acid, Luteolin, and Syringic Acid, the highest values reached at a concentration of 900 ppm in all these compounds. These values were 110.255, 78.644, 88.722, and 69.277 mg/L, respectively.

Conclusion

The results of this study showed that adding biocarbon to the culture medium increased the amount of protein, fat, carbohydrates, and phenolic compounds in *Chlorella vulgaris* algae. The highest amount of these compounds was observed at a concentration of 900 mg/L and at a harvest time of 14 days. Therefore, biocarbon can improve the growth and production of beneficial compounds in this alga.

Keywords: biocarbon, carbohydrate, lipid, phenolic acid, protein

Paper Type: Research Paper.

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Introduction

Algae are considered a diverse group of organisms ranging from unicellular, such as microalgae or phytoplankton, to multicellular, such as macroalgae or filamentous algae (Sambusiti et al., 2015). *Chlorella vulgaris* is a microalga that is a promising candidate for solving many problems due to its ease of cultivation, rapid growth, absorption of carbon dioxide, and production of oxygen during its growth, as well as its production of many primary and secondary compound. Algae are regarded as a promising biomass raw material for fuels, chemicals, and sustainable materials. This is because these algae are mainly composed of lipids, carbohydrates,

and proteins (Sun et al., 2022). *Chlorella vulgaris* is considered a protein-rich microalga having potential applications in the food and feed industries. Microalgae have been consumed in Asian countries for centuries and are regarded a healthy food because of their high content of vitamins, minerals, proteins, and fiber, plus many other bioactive compounds (Niccolai et al., 2019). The population of the world is continuously increasing with a significant increase in food demand. The global population is expected to increase by 50% by the end of 2050. Thus, it is essential to increase food production to meet the increasing rising nutritional needs (Robertson et al., 2023). Chlorella is thought to be a sustained and promising source of vital nutrients and bioactive substances. However, the presence of a cell wall that is predominantly composed of insoluble carbohydrates like cellulose and chitin-like polymers limits the bioavailability of these nutrients. (Bhatia et al., 2022). Accordingly, Chlorella algae are considered an important species with great potential as a staple food because not only its content of high-value protein but also its high value of energy, lipid content, minerals, and vitamins (Tejano et al., 2019). Biochar is regarded a carbon-rich solid material formed from organic residues through the thermal decomposition. Biochar has made significant achievements in increasing the agricultural productivity and decreasing the bioavailability of environmental pollutants. Thus, it has become a value-added product that supports the bioeconomy, i.e., the exploration and exploitation of biological resources by using biotechnology of producing new biological products having an economic value (Oni et al., 2019). Biochar has granted an increasing attention due to its unique characteristics, such as its high carbon content, capacity of cation exchange, large qualitative surface area, stable construction, and its use as a catalyst and activator for various compounds (Wang and Wang, 2019). Because of the increase of consumers' preference for choosing products with natural ingredients, the food industry has directed scientific research in this direction. Algae are regarded an attractive choice for research as they can synthesize a range of secondary compounds called phenolic compounds, which are associated with promising biological properties and activities (Jimenez-Lopez et al., 2021). Flavonoids are among the most valuable phytochemicals and have been identified in many microalgae and macroalgae. The high growth rate, minimal nutrient and growth requirements, availability of arable land, and rich metabolic profile make microalgae an excellent source of new anticancer compounds such as flavonoids (Ferdous and Balia Yusof, 2021). Rutin is considered as one of the nutrient flavonoid compounds. It found frequently in 70 plant species and it is also known as vitamin p (Prasad and Prasad, 2021). Rutin is widely found in cyanobacteria, which have shown high activity in removing free radicals (Singh et al., 2017). Regarding to Caffeic acid, it acts as an antioxidant and anticancer agent and it exhibits a significant antibacterial activity. In addition, it displays antiviral activity, anti-inflammatory, and immune-boosting capabilities (Espíndola et al., 2019). Luteolin which is a flavonoid found in

many fruits, vegetables, and herbs has been identified as having numerous biological activities, including anti-inflammatory, anti-diabetic, and anti-cancer properties. Extensive research has been conducted on Luteolin's anti-cancer properties in various cancers. It was connected to its ability to inhibit tumor growth by targeting cellular processes such as apoptosis, angiogenesis, migration, and cell cycle progression (Çetinkaya and Baran, 2023). Syringic acid is another flavonoid compound, and there are numerous vitro and vivo studies that have evaluated its pivotal effects on oxidative stress and inflammatory parameters. It is considered a prominent compound that may help address health problems associated with modern diseases (Bartel et al., 2023). A study by Mohammad et al. (2025) showed that biochar contains porous structures, large surface areas, and a variety of surface functional groups. In latest years, most research has focused on the effect of the application of biochar to the cultivation of annual crops and plants in general. The results have varied in terms of positive and negative effects depending on the source of the biochar as well as its concentration used. As for its effect on algae, studies are characterized by weakness in applying the use of biochar and its addition to the culture medium and observing its effect on primary and secondary metabolites. The aim of this study was to detect the active compounds in the algae *Chlorella vulgaris* and study the effect of the production of primary and secondary metabolites under the influence biochar in algae Chlorella vulgaris.

Materials and methods

The experiment was conducted in the Plant Cell and Tissue Culture Laboratory, Department of Biology, College of Education for Pure Sciences, University of Diyala, under sterile conditions using a sterile atmosphere transfer table. Samples were stored in a growth room at 25 ± 25°C under an 8/16-hour light-dark cycle with a light intensity of 3000 lux. Samples were put on a vibrating shaker to ensure continuous movement of the samples.

Preparation of culture media: The Bg11 culture medium was prepared by taking 1.6 g. of the culture medium by using a sensitive balance. Next, it was dissolved in 1L. of distilled water in a 1000 cm^3 flask. After that, the flask was placed in a magnetic hotplate to dissolve the culture medium. Then, the flask was covered with cellophane to sterilize the culture medium using an autoclave. The conditions of autoclave conditions (temperature was 118°C and pressure was 121 bar). The sterilization took 15 minutes, and then the culture medium was left to cool at the room temperature. Then, the medium was distributed into 3 replicates for each treatment and each phase.

Preparation of the isolate: Only 900 mL of the culture medium was taken and added to each replicate and the volume was completed to be 1 liter with the algal isolate.

Preparation of biocarbon: Biocarbon was prepared by dissolving 0.025 g/L in 1 mL of hydrochloric acid and then 250 mL of distilled water was added. Before using it, biocarbon was left for 24 hours in the refrigerator at the required concentrations for the experiment 300, 600, and 900 mg/L, which were then added to the culture media containing the algal isolate.

Cell harvesting: Chlorella cells were collected three times during the study, at each developmental stage of growth for each group in the culture vessel. First harvest was at 7 d, followed by the second at 14 and third after to 21 days (Jawad, 1982).

Protein estimation: The Kjeldahl method was used to estimate the protein content in the samples. A known weight of the sample (approximately 5 g) was placed in a flask. Only 25 mL of concentrated sulfuric acid was added, along with an appropriate amount of a mixture of potassium sulfide and copper sulfide. Digestion was carried out by heating the contents. After digestion, the mixture was converted into a clear, pale blue liquid. Next, this liquid was quantitatively transferred to the Kjeldahl distillation flask containing a solution of 40% concentrated sodium hydroxide. The distillation flask was connected to a condenser ending in a test tube immersed in a receiving flask containing a known volume of 20% boric acid which was added drops of methyl red indicator and bromocresol blue dye. Then the flask was heated to reached approximately 25 mL of the collected distillate was collected and it the liquid was centrifuged with 0.1 N hydrochloric acid. After that, a control solution (Blanc) was prepared from chemical materials above except the pattern. The protein percentage is calculated using the following equation (van Dijk and Houba, 2000):

$$\text{Protein Percentage \%} = \frac{(\text{Volume of HCl consumed} \times \text{Normality} \times 0.014 \times 6.25)}{\text{Sample weight} \times 100}$$

Lipid estimation: The lipid content was determined by taking 10 g of the dried sample which was placed on filter paper and a roll, then placed in the thimble of a lipid extraction apparatus (Soxholet). The flask was weighed, then hexane (250 mL) was added. The extraction procedure went on for around five hours. The solvent was then extracted from the device, and the flask was taken out and put in an electric oven set to 80°C for 30 minutes to ensure that any leftover solvent evaporated and the lipid was preserved. Then, it was removed from the oven and left to cool. Finally, the flask was weighed, and the lipid percentage was determined according to the following equation (AOAC, 1995):

$$\text{Lipid percentage \%} = \frac{\text{Weight of flask before extraction} - \text{Weight of flask after extraction}}{\text{Weight of sample} \times 100.}$$

Carbohydrate analysis: The total carbohydrate content was estimated by weighing 0.2 g of the sample to be measured and adding 25 mL of perchloric acid 1N. The mixture was placed in a test tube, which was then placed in a water bath at 60°C for 30 minutes. The sample was then

filtered using filter paper. Only 1 mL of the filtrate was taken and mixed with 9 mL of distilled water to bring the total volume to 10 mL in a volumetric flask. Only 1 mL of this filtrate was then taken and mixed with 5% phenol plus 5 mL of concentrated sulfuric acid. The mixture was allowed to cool and measured at a wavelength of 490 nm using a spectrophotometer. Several glucose concentrations (1, 2, 3, 4, and 5) were prepared, and the absorbance readings above were recorded (to construct a calibration curve). The absorbance reading was then taken. The sample is projected onto the calibration curve, and the concentration is identified using the following equation (Hedge and Hofreiter, 1962):

$$X = \frac{\text{(Volume of acid) x (Final volume of sample) x Concentration from the titration curve}}{\text{(Weight of sample x 1000)}} \times 100\%$$

Measurement of flavonoid acids: Three g sample powdered plant is extracted with 12 mL chloroform, under stirring and for a period of 8 hours at room temperature. The extract was sonicated for 15 min. It was added to 100 mL butanol and the mixture was transferred to a separation funnel. The polar organic layer (butanol) was collected, and evaporated using a rotary evaporator apparatus to give dry extract. The procedure was carried out three times to have enough sample the analysis.

HPLC condition: Sample were analyzed by high performance liquid chromatography (HPLC) model SYKAM (Germany). The mobile phase was Methanol:D.W.:formic acid as 70:25:5. The column was C18-ODS (25 cm × 4.6 mm) and detector UV 280 nm at flow rate 1.0 mL /min.

Design and analysis of the experiment: Three replicates of each treatment were used in the CRD experiment design. SPSS software was used to analyze the data at a significance level of 0.05.

Results and discussion

The results showed that the highest protein concentration was 61.666 mg/L and was recorded with the addition of 900 ppm biocarbon (Table 1). This indicates that the concentration significantly enhanced protein production. In contrast, the lowest protein concentration was 53.455 mg/L that was observed in the control treatment. Concerning the effect of different harvest days on protein concentration, the highest protein value was 60.025 mg/L observed in the second growth stage. This indicates that biocarbon is most effective during this stage. As for the interaction between concentration and cell harvest days, the highest protein value was 64.00 mg/L. This value was observed at a concentration of 900 ppm in the second growth stage and demonstrated a positive interaction between the high biocarbon concentration and the algal

growth stage. Conversely, the lowest protein concentration was 52.533 mg/L and was observed in the control treatment after 7 days of cell harvesting.

Table 1. The impact of various biocarbon concentrations (ppm), growth phases, and their interactions on *Chlorella vulgaris* protein concentration

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	52.533 ^{k}	55.433 ^{h}	57.233 ^{f}	59.133 ^{e}	39.916 ^{c}
14 Days	54.733 ^{i}	58.833 ^{e}	62.533 ^{b}	64.000 ^{a}	53.570 ^{a}
21 Days	53.100 ^{j}	56.433 ^{g}	60.833 ^{d}	61.866 ^{c}	48.391 ^{b}
Effect of Biocarbon	**53.455 ^{d}**	**56.900 ^{c}**	**60.200 b**	**61.667 ^{a}**	

Values are presented as means. Different superscript letters indicate significant differences among treatments at $p < 0.05$ according to the statistical test used.

The results showed that the highest lipid concentration was 9.155 mg L^{-1} at a biocarbon concentration of 900 ppm, while the lowest value was 6.510 mg L^{-1} in the control group (Table 2). Regarding the results of different growth stages, a highest value was 8.540 mg L^{-1} after 14 days of growth, whereas the results for the interaction between biocarbon and different growth stages showed that the highest value was 9.886 mg L^{-1} at a concentration of 900 ppm after 14 days of growth and the lowest value was 6.153 mg L^{-1} in the control group after 7 days of growth.

Table 2. Impact of varying development stages, biocarbon concentrations, and their interplay on *Chlorella vulgaris* lipid concentration

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	6.153^{k}	7.246^{h}	7.800^{g}	8.400^{e}	7.400^{c}
14 Days	6.830^{i}	8.000^{f}	9.466^{b}	9.866^{a}	8.540^{a}
21 Days	6.546^{j}	7.400^{h}	8.833^{d}	9.200^{c}	7.995^{b}
Effect of Biocarbon	**6.510^{d}**	**7.548^{c}**	**8.700^{b}**	**9.155^{a}**	

Values are presented as means. Different superscript letters indicate significant differences among treatments at $p < 0.05$ according to the statistical test used.

Table 3 shows that the highest carbohydrate concentration was 18.362 mg L^{-1} at a concentration of 900 ppm, while the lowest was 15.538 mg L^{-1} in the control group. Regarding to the results of different growth stages, the highest concentration was 17.741 mg L^{-1} after 14 days of growth. The results of interaction indicate that the highest concentration was 19.000 mg L^{-1} at a concentration of 900 ppm after 14 days of growth, while the lowest value was 15.22 mg L^{-1} in the control group after 7 days of growth.

Table 3. Impact of varying biocarbon concentrations, growth phases, and their interplay on *Chlorella vulgaris* carbohydrate content

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	15.223^{i}	16.023^{g}	16.806^{e}	17.670^{c}	16.430^{c}
14 Days	15.843^{gh}	17.243^{d}	18.880^{a}	19.000^{a}	17.741^{a}
21 Days	15.550^{hi}	16.416^{f}	17.970^{c}	18.416^{b}	17.088^{b}
Effect of Biocarbon	**15.538^{d}**	**16.561^{c}**	**17.885^{b}**	**18.362^{a}**	

Values represent mean measurements. Means followed by different superscript letters indicate significant differences among treatments at $p < 0.05$.

Table (4) showed that the highest Rutin concentration was (110.255 mg L^{-1}) at a concentration of 900 ppm, while the lowest value was (60.311 mg L^{-1}) in the control group. The highest Rutin concentration at different growth stages was (99.725 mg L^{-1}) after 14 days of growth. The highest Rutin concentration was (120.800 mg L^{-1}) at 900 ppm after 14 days of growth and the lowest was (52.900 mg L^{-1}) in the control group after 7 days of growth.

Table 4. The concentration of rutin in *Chlorella vulgaris* affected by varying biocarbon concentrations, growth stages, and their interactions

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	52.900^{l}	74.200^{i}	89.833^{g}	98.833^{e}	78.941^{c}
14 Days	67.100^{j}	95.200^{f}	115.800^{b}	120.800^{a}	99.725^{a}
21 Days	60.933^{k}	80.733^{h}	107.733^{d}	111.133^{c}	90.133^{b}
Effect of Biocarbon	**60.311^{d}**	**83.377^{c}**	**104.455^{b}**	**110.255^{a}**	

Values represent mean measurements. Means followed by different superscript letters indicate significant differences among treatments at $p < 0.05$.

Table 5 showed that, in contrast to the lowest value, which was 43.144 mg L^{-1} in the control treatment, the highest value of caffeine was 78.644 mg L^{-1} at a concentration of 900 ppm. The highest value, based on the data for the various growth phases, was 71.629 mg L^{-1} following 14 days of growth. After 14 days of growth, the interaction's greatest value was 88.400 mg L^{-1} at a concentration of 900 ppm, while the control treatment's lowest value was 38.050 mg/L after 7 days of growth.

Table 6 revealed that at a concentration of 900 ppm, the highest value of luteolin was 88.722 mg/L, while the lowest value was 46.416 mg/L in the control group. However, after 14 days of growth, the results of the several growth stages showed that the maximum value was 79.100 mg/L. The maximum concentration of the interaction was 97.433 mg/L at a concentration of 900 ppm

after 14 days of growth, whereas the lowest value for the control group was 42.050 mg/L after 7 days of growth.

Table 5. The concentration of caffeine in *Chlorella vulgaris* affected by varying biocarbon concentrations, growth phases, and their interactions.

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	38.050^{h}	52.050^{g}	60.800^{f}	69.233^{de}	55.033^{c}
14 Days	49.333^{g}	65.733^{ef}	83.050^{ab}	88.400^{a}	71.629^{a}
21 Days	42.050^{h}	62.533^{f}	73.030^{cd}	78.300^{bc}	63.979^{b}
Effect of Biocarbon	**43.144^{d}**	**60.105^{c}**	**72.294^{b}**	**78.644^{a}**	

Values represent mean measurements. Means followed by different superscript letters indicate significant differences among treatments at $p < 0.05$.

Table 6. The concentration of luteolin in *Chlorella vulgaris* affected by varying biocarbon concentrations, growth stages, and their interactions

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	42.050^{k}	55.633^{h}	69.200^{f}	77.900^{d}	61.204^{c}
14 Days	50.800^{i}	71.900^{e}	96.266^{a}	97.433^{a}	79.100^{a}
21 Days	46.400^{j}	63.300^{g}	87.400^{c}	90.833^{b}	71.958^{b}
Effect of Biocarbon	**46.416^{d}**	**63.577^{c}**	**84.300^{b}**	**88.722^{a}**	

Values represent mean measurements. Means followed by different superscript letters indicate significant differences among treatments at $p < 0.05$.

The results showed that the highest syringic acid level was 69.277 mg/L at a concentration of 900 ppm, while the lowest level was 30.611 mg/L in the control group (Table 7). The results at different growth stages showed that the highest level was 60.908 mg/L after 14 days of growth. The results of the interaction showed that the highest level was 79.300 mg/L at a concentration of 900 ppm after 14 days of growth, while the lowest level was 24.833 mg/L in the control group after 7 days of growth.

The significance and uniqueness of this study are because it is the first study that adds biocarbon as a nutrient and stimulant source for growing algae in enclosed environments. The positive effect of biocarbon on protein concentration may be attributed to its role in enhancing protein degradation through increasing the activity of gene expression of three proteins responsible for protein breakdown. These proteins are serine protease, aspartic acid protease, and cysteine protease. In addition, it was found that biocarbon enhances the expression level of two genes involved in protein synthesis. They encode the small and large subunits of the rubisco protein (Noguera et al., 2012). But after 14 days of growth, this may be attributed to the increase

of metabolic activity at this growth stage when cells are actively proliferating and producing proteins in response to improved environmental conditions.

Table 7. The content of syringic acid in *Chlorella vulgaris* affected by varying biocarbon concentrations, development stages, and their interactions

Days	Control	300 ppm	600 ppm	900 ppm	Effect of Days
7 Days	24.833^{n}	39.233^{i}	49.233^{g}	59.233^{e}	43.133^{c}
14 Days	36.200^{l}	53.100^{f}	75.033^{b}	79.300^{a}	60.908^{a}
21 Days	30.800^{m}	43.800^{h}	63.833^{d}	69.300^{c}	51.933^{b}
Effect of Biocarbon	**30.611^{d}**	**45.377^{c}**	**62.700^{b}**	**69.277^{a}**	

Values represent mean measurements. Means followed by different superscript letters indicate significant differences among treatments at $p < 0.05$.

Khan et al. (2023) also observed that the addition of biocarbon increased the synthesis of total soluble proteins, free amino acids, and proline. These results may be explained by the role of biochar in photosynthesis which is one of the most important physiological processes. The use of biochar improves photosynthesis through chlorophyll formation and electron transport (Manolikaki and Diamadopoulos, 2019). In another study Liu et al. (2023) discovered that adding biochar increases antioxidant activity and electron transport. Plus, it increased the efficiency of photosynthesis. Biochar is considered an essential and important compound in modern agriculture. Studies have proved its positive effect on lipid accumulation in various plant species by increasing lipid concentration within tissues. Studies have demonstrated that it contains rich compounds contributing to improving the lipid composition of plants; thereby this improves their nutritional value and increases the diverse production of lipid compounds derived from renewable natural sources. Thakkar (2021) demonstrated that biochar plays an important role in increasing chlorophyll content and the rate of photosynthesis, which in turn helps increase the production of active primary compounds such as lipids (Khan et al., 2023). These findings could account for biochar being able to induce situation favoring nutrient uptake, release biologically active compounds that enhanced the growth and development of a plant crop and pathogen suppression. Therefore, biochar can improve the growth, health and disease resistance of plants (Stopa et al., 2023). Carbohydrates are considered an important criterion for assessing nutritional value, which depends on several factors such as the growth stage at which the harvest is carried out, environmental factors plus nutrients (Mak-Mensah et al., 2023). Biochar has been used as a sustainable supplement to mitigate environmental risks and improve plant growth and soil properties. It has been found that adding biochar increased total nitrogen, phosphorus, and potassium in the soil (Guo et al., 2023). Many practices like adding biochar have been used to

affect the properties of growth environments. Given that polyphenolic compounds, such as flavonoids, are responsible for the response to stress caused by unfavorable environmental conditions (Regmi et al., 2023). It has been found that adding biochar to the soil significantly increase the levels of several amino acids. This may be attributed to the increased nutrient availability and, consequently, increased accumulation of secondary metabolites (Liu et al., 2023).

Conclusions: The results showed that increasing the concentration of biochar effectively contributes to the productivity of metabolic compounds. This treatment enhanced the increase in primary metabolites (proteins, fats, carbohydrates) along with an increase in secondary metabolites of phenolic acids. Harvesting cells after 14 days of algal growth was found to have a positive effect on increasing the concentration of both primary and secondary metabolites. Therefore, it can be concluded that biocarbon can improve the growth and production of beneficial compounds in this alga.

Author contributions

M. M. I. and F.Q.A designed the study. Z.G.F performed the experiments, analyzed the data and wrote the paper with input from all authors.

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Data Availability

The data that contributes to the results of this study is provided by the researcher responsible for the request.

Ethical Considerations

No human or animal participants were used in this study. All laboratory procedures were performed according to standard guidelines.

Conflict of Interest

The researchers declare that there is no conflict of interest regarding the publication of this paper.

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پویایی متابولیت‌های اولیه و ثانویه در *Chlorella vulgaris* در معرض تنش زیستی و دوره‌های مختلف برداشت

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چکیده

هدف: *Chlorella vulgaris* یک ریزجلبک است که به دلیل سهولت کشت، رشد سریع، توانایی جذب دی‌اکسید کربن و تولید اکسیژن در طول رشد، و همچنین تولید ترکیبات اولیه و ثانویه متعدد، گزینه‌ای امیدوارکننده برای حل بسیاری از مسائل به شمار می‌رود. هدف این مطالعه بررسی اثر غلظت‌های مختلف بیوکربن، زمان‌های متفاوت برداشت سلول‌ها و برهم‌کنش آن‌ها بر جلبک *Chlorella vulgaris* بود. همچنین میزان پروتئین، لیپید، کربوهیدرات و اسیدهای فنولی نیز اندازه‌گیری شد.

مواد و روش‌ها: این آزمایش در آزمایشگاه کشت بافت گیاهی دانشگاه دیاله و تحت شرایط استریل انجام شد. جلبک‌ها در محیط کشت BG11 در دمای حدود ۲۵ درجه سانتی‌گراد و دوره نوری ۸–۱۶ ساعت رشد داده شدند. بیوکربن با غلظت‌های ۳۰۰، ۶۰۰ و ۹۰۰ میلی‌گرم بر لیتر به محیط کشت اضافه شد. نمونه‌ها در روزهای ۷، ۱۴ و ۲۱ جمع‌آوری گردیدند. سپس مقدار پروتئین با روش کجلدال، چربی با دستگاه سوکسله، کربوهیدرات با روش فنل–اسید سولفوریک و ترکیبات فلاونوئیدی با استفاده از HPLC اندازه‌گیری شد. آزمایش در قالب طرح کاملاً تصادفی با سه تکرار انجام گرفت و داده‌ها با نرم‌افزار SPSS در سطح معنی‌داری ۰/۰۵ تحلیل شدند.

نتایج: نتایج نشان داد که با افزایش غلظت بیوکربن در محیط کشت، مقادیر ترکیبات مورد بررسی افزایش یافت و بیشترین مقادیر به‌ترتیب ۶۱/۶۶۶، ۹/۱۵۵ و ۱۸/۳۶۲ میلی‌گرم بر لیتر در غلظت ۹۰۰ پی‌پی‌ام مشاهده شد. از نظر زمان برداشت سلول‌ها، بیشترین مقادیر پس از ۱۴ روز از افزودن بیوکربن به محیط کشت به دست آمد که به‌ترتیب برای پروتئین، لیپید و کربوهیدرات برابر با ۶۰/۰۲۵،

۸/۵۴۰ و ۱۷/۷۴۱ میلی‌گرم بر لیتر بود. همچنین در بررسی اسیدهای فنولی شامل روتین، اسید کافئیک، لوتئولین و اسید سیرینگیک، بیشترین مقادیر در غلظت ۹۰۰ پی‌پی‌ام مشاهده شد که به‌ترتیب برابر با ۱۱۰/۲۵۵، ۷۸/۶۴۴، ۸۸/۷۲۲ و ۶۹/۲۷۷ میلی‌گرم بر لیتر بودند.

نتیجه‌گیری: نتایج این مطالعه نشان داد که افزودن بیوکربن به محیط کشت باعث افزایش میزان پروتئین، چربی، کربوهیدرات و ترکیبات فنولی در جلبک *Chlorella vulgaris* می‌شود. بیشترین مقدار این ترکیبات در غلظت ۹۰۰ میلی‌گرم بر لیتر و زمان برداشت ۱۴ روز مشاهده شد. بنابراین، بیوکربن می‌تواند رشد و تولید ترکیبات مفید در این جلبک را بهبود بخشد.

کلمات کلیدی: اسید فنولی، بیوکربن، پروتئین، کربوهیدرات، لیپید

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