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Effect of different trophic cultures on the amount of total carbohydrate and chlorophyll of *Oscillatoria* sp.

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ABSTRACT

Cyanobacteria (blue-green microalgae) is a gram-negative prokaryotic autotroph found in natural waters that plays a pivotal role in biochemical cycles. The present investigation proposed to study the potential of using different concentrations of glucose as the carbon substrate to produce microalgal biomass and biochemical components, such as photosynthetic pigments and total carbohydrates (C.H.) by *Oscillatoria* sp. The cyanobacteria were collected, and the isolated colony was found to be *Oscillatoria* sp., and it was grown in BG-11 medium for mass cultivation. Then, the centrifuged biomass was weighed and used to extract bioactive compounds. *Oscillatoria* sp. cells were cultured in three different tropic cultures (phototrophic, heterotrophic and mixotrophic) under controlled laboratory conditions with continuous light illumination or unillumination and aeration. Chl–a and total C.H. contents were also evaluated after 120 hrs. The recorded optical density of *Oscillatoria* was increased from 0.6798 ±0.01 at 660 nm and 0.5847 ±0.01 at 750 nm after 24 hrs to 1.2174 ±0.002 at 680nm and 1.0243 ±0.01 at 730nm at the end of 120hrs of the experiment. According to analysis results, the mean amount of Chl-a and Total C.H. of *Oscillatoria* sp. biomass was determined as 0.5132 µg L^{-1} and 3.5715 mg mL^{-1} under the phototrophic culture (absence of glucose), respectively. Under the mixotrophic culture (presence of light), the experimental results showed that the chl-a content was calculated as 0.1770, 0.3380 and 0.7098 µg L^{-1}. In contrast, the total C.H. was calculated as 3.6150, 7.9129 and 11.3191 mg mL^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively. Under the heterotrophic culture (absence of light), the results showed that the chl-a content was 0.2366, 0.2456 and 0.2346 µg L^{-1} while the total C.H. was 4.2969, 8.0990 and 11.5861 mg m L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively. The experimental results showed that the total C.H. content was increased from 3.5715 to 11.58 61 mg mL^{-1} in the heterotrophic (the absence of light and the presence of 10 g L^{-1} glucose) BG-11 culture conditions. The chlorophyll-a content was increased from 0.1770 µg L^{-1} to 0.7098 µg L^{-1} in the mixotrophic (the presence of glucose and light) BG-11 culture conditions. As a result of the experiment, it was determined that the most suitable culture in terms of total carbohydrate and growth rate was mixotrophic and heterotrophic BG-11 (10 g L^{-1} glucose) culture condition, and in terms of chl-a was mixotrophic culture (10 g L^{-1} glucose).

Keywords: Cyanobacteria, *Oscillatoria*, Glucose, Mixotrophic cultivation, Heterotrophic cultivation

Introduction

Microalgae are photosynthetic organisms found in pelagic and benthic environments (cyanobacteria, diatoms, dinoflagellates, and green algae) (Bhuyar et al., 1990; Abad et al., 2011; Bhuyar et al., 2020). They consist of various biomolecules, majorly including lipids, proteins, pigments, chlorophyll, and carbohydrates, which are found in the form of cellulose and soluble polysaccharides such as starch or glycogen (Paroz & Izquirerdo, 2011).

Cyanobacteria, found in many different habitats, from fresh to marine, hyper-saline, and terrestrial ecosystems, are gram-negative prokaryotic autotrophs that use chlorophyll for photosynthesis. In addition to photosynthesis, cyanobacteria, an incredible ancient group of prokaryotic organisms, are wealthy in numerous treasured compounds like pigments (astaxanthin, lutein, phycobiliprotein), antibiotics, vitamins, and essential nutrients (proteins, carbohydrates, and lipids) (Lau et al., 2015).

Oscillatoriaceae species (*Spirulina* sp., *Oscillatoria* sp., *Phormidium* sp., *Lyngbya* sp.) produce many pharmaceutical and nutraceutical ingredients with varying bioactivities, including antibacterial, antifungal, antioxidant, antialgal, antiviral, anticancer, and anti-inflammatory effects. This is one of the most important orders in cyanobacteria (Singh et al., 2022; Sultan et al., 2016).

A transition from phototrophic growth to mixotrophic growth can be observed in many species and genera of microalgae. Some microalgae species like *Chlorella vulgaris* (Mitra et al., 2012), *Haematococcus pluvialis* (Kobayashi et al., 1992), *Spirulina platensis* (Marquez et al., 1993), *C. sorokiniana* (Wang et al., 2012), *Botryococcus braunii* (Zhang et al., 2011), and *C. zofingiensis* (Li et al., 2011) have been observed under autotrophy, heterotrophy, and mixotrophy conditions. Many algal organisms can use either metabolic process (autotrophic or heterotrophic) for growth, meaning that they can photosynthesise and ingest prey or organic materials (Lau, 2015). The ability of mixotrophism to process organic substrates means that cell growth is not strictly dependent on photosynthesis. Therefore, light energy is not a limiting factor for growth and light, or organic carbon substrates can support the alga growth. Hence, there is less biomass loss during the dark phase. Mixotrophic and heterotrophic microalgae cultivation provides higher biomass and lipid productivities than cultivation under photoautotrophic conditions; the cost of the organic carbon substrate is estimated to be about 80% of the total cost of the cultivation medium (Choi & Lee, 2015).

In this study, it was investigated in which trophic culture environment *Oscillatoria* cells produce more chlorophyll and carbohydrates. The present work, therefore, investigates the effects of different trophic conditions (phototrophic, heterotrophic and mixotrophic) and different glucose substrate concentrations on total carbohydrate, chlorophyll-a total protein contents of *Oscillatoria* sp. studied under laboratory conditions. Optical density, carbohydrate and chlorophyll-a content of *Oscillatoria* cells were measured by spectrophotometric method. *Oscillatoria* sp. cells were cultured under controlled laboratory conditions with continuous light illumination and aeration. Optical density, chl-a and carbohydrate contents were also evaluated after 120hrs.

Materials and Methods

Sample Collection Followed by Identification

The samples were collected from Gediz River (38°39′40.7″ N, 27°18′44.2″ E), Manisa, Türkiye in August 2023. The algae samples were collected with microalgae by plankton net (55 µm mesh) and brought to the laboratory. Serial dilution was carried out to get isolated colonies. The algae were observed and identified directly under the microscope (Olympus Cover-015) between lam and lamel. The photographs were taken using normal microphotography techniques. Current literature sources were used for the determinations. The morphological observations referred to the blue-green algae group that belongs to *Oscillatoria* sp, as shown in Figure 1. (Desikachary, 1959; Komárek & Anagnostidis, 2005; Guiry & Guiry, 2015).

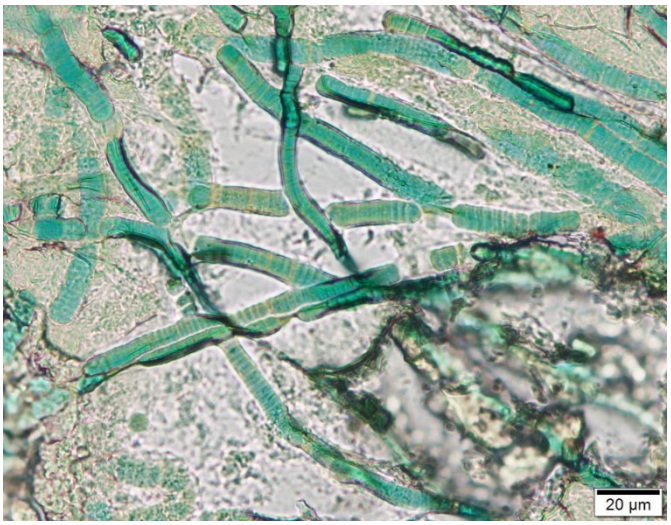

Figure 1. *Oscillatoria* sp. microalgae under microscopic view (x40)

Cultivation

A standard initial inoculum of the isolated algae was inoculated to culture flasks (200 mL each) that contained 100 mL of BG-11 medium, which consists of macronutrients (1.5 g $NaNO_3$ L^{-1}, 0.04 g K_2HPO_4 $3H_2O$ L^{-1}), inorganic salts (0.036 g $CaCl_2.7H_2O$ L^{-1}, 0.075 g $MgSO4.7H_2O$ L^{-1}, 0.02 g Na_2CO_3 L^{-1}), pH conditioners (0.001 g Na_2EDTA L^{-1}, 0.006 g $C_6H_8O_7$ L^{-1}, 0.006 g $C_6H_8O_7{\cdot}xFe^{3+}{\cdot}yNH_3$ L^{-1}), and trace elements (0.222 g $ZnSO4.7H_2O$ L^{-1}, 1.81 g $MnCl.4H_2O$ L^{-1}, 0.390 g Na_2MoO_4. $2H_2O$ L^{-1}, 0.079 g $CuSO_4.5H_2O$ L^{-1}, 2.86 g H_2BO_3 L^{-1}, and 0.0494 g $Co(NO_3)_2.6H_2O$ L^{-1}).

The culture media were sterilised by autoclaving at 121 °C and 1 bar for 15 min and incubated at 26 ± 1ºC under 16:8 h photoperiod (light intensity = 40 µE/m2 S), with aeration (1.2 L min^{-1}) and magnetic stirring (110 rpm). The pH value was adjusted to 6–7 using 1 M NaOH and 1 M HCI (Vijayabaskar & Shiyamala, 2011; Bhuyar et al., 2019). The growth of algae and biomass concentration was monitored by measuring optical density at a wavelength of 660 nm and 730 nm for 20 days. After the lag phase, the algal cells got into their logarithmic growth phases.

Experimental Conditions

Phototrophic culture (in the presence of light and absence of glucose)

Oscillatoria adapted to phototrophic growth conditions as described in the cultivation section. Cultures in the log (exponential) phase were used in this study. The culture was transferred as inoculum into 500 ml Erlen Mayer containing 200 ml of medium and 50 ml of biomass for 120 h under 16:8 h photoperiod conditions. Experiments were conducted in sets of three. The temperature and pH were 25-26°C and between 6.14 ± 0.07 and 6.87 ± 0.03, respectively. Sterile and humidified air was provided by a pump to supply enough oxygen and distribute it homogeneously in the incubator (Biosan ES-20/60). The culture was continuously mixed using a magnetic stirrer.

Heterotrophic culture (in the absence of light and the presence of glucose)

Oscillatoria biomass was cultivated in BG-11 medium using 2.5, 5 and 10 g glucose L^{-1} as the initial glucose concentration in the dark to identify the effect of different glucose concentrations on cell growth, chl-a and total CH—amount of the microalgae.

Mixotrophic culture (in the presence of light and glucose)

A series of experiments were conducted to assess the influence of glucose concentration (2.5, 5 and 10 g glucose L^{-1}) on mixotrophic microalgae growth in addition to chl-a and total CH. Amount of biomass.

Analysis of the Total Carbohydrate Contents (mg mL^{-1})

Total carbohydrate contents were measured using the phenol-sulfuric acid assay, a simple and rapid colourimetric method to determine total carbohydrates, and the d-glucose concentration scale was used to construct the standard curve by DuBois et al. (DuBois et al., 1956). Briefly, a 5% (w/v) phenol solution was prepared in distilled water. 50 µL of test samples and 2 mL of concentrated sulfuric acid were added to 50 µL of a phenol solution. The mixture was stirred for 30 min. 1 mL aliquots of the cultures were used to quantify spectrophotometrically at 490 nm.

Analysis of the Chlorophyll Content (µg L^{-1})

The spectrophotometric method, measured by the absorbance at 630 nm, 645nm, 665 nm, and 750 nm using a spectrophotometer with 90% acetone as blank, was used for the determination of pigment concentrations (Parsons & Strickland,1963). Briefly, 10 mL of culture was filtered using GF/C filters. An aliquot of the sample was centrifuged at 12000 rpm for 5 min, and the supernatant was discarded. The pellet was suspended in 10 mL of boiling acetone at 4°C, stored in the dark for 24 h, and measured using a spectrophotometer.

Determination of Dry Weight

A definite volume (10 mL) of algal suspension was filtered through a cellulose acetate filter membrane (47mm in diameter, 0.22 µm in pore size) and dried overnight in an oven at 105°C. Data were given as mg mL^{-1} algal suspension.

Growth Estimation

Growth was estimated in each sample by measuring the biomass turbidity of SP homogenised suspension at wavelengths 660 nm and 730 nm using a spectrophotometer (LW UV-200-RS); it was expressed in dry mass per litre of suspension (Seely et al., 1972).

Results and Discussion

The Analysis Results of Chl-a content (µg L^{-1}) of Biomass

Tables 1-3 show the effects of initial 2.5, 5, and 10 g L^{-1} glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria*

sp. during the different trophic incubation periods. The chlorophyll-a content (µg L^{-1}) of treated microalgae cells increased significantly with increased glucose concentrations under the mixotrophic culture (M.C.) conditions. The chl-a contents of cells were affected significantly, especially at 5 and 10 g L^{-1} glucose concentrations.

According to analysis results, the mean amount of Chl-a of *Oscillatoria* sp. biomass was determined as 0.5132 µg L^{-1} under the phototrophic culture (absence of glucose). Under the mixotrophic culture (presence of light), the experimental results showed that the chl-a content was calculated as 0.1770, 0.3380 and 0.7098 µg L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 2-4). Under the heterotrophic culture (absence of light), the results showed that the chl-a content was 0.2366, 0.2456 and 0.2346 µg L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively.

Table 1. The effect of initial 2.5 g/L glucose concentration on the chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5079±0.002
2. Heterotrophic	0.2366±0.004
3. Mixotrophic	0.1770±0.001

Table 2. The effect of initial five g/L glucose concentration on the chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5201 ±0.002
2. Heterotrophic	0.2456 ±0.001
3. Mixotrophic	0.3380 ±0.002

Table 3. The effect of initial 10 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5116 ±0.002
2. Heterotrophic	0.2346 ±0.001
3. Mixotrophic	0.7098 ±0.001

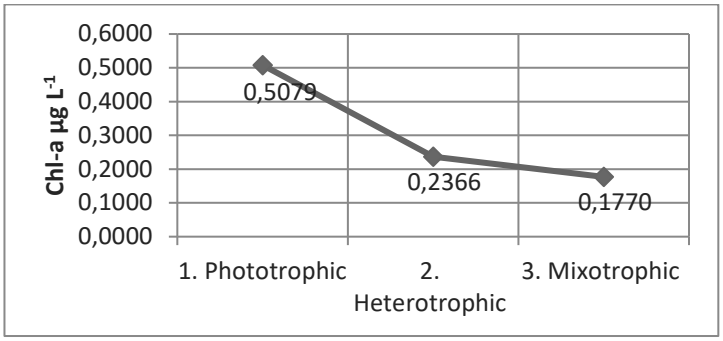

Figure 2. The effect of initial 2.5 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

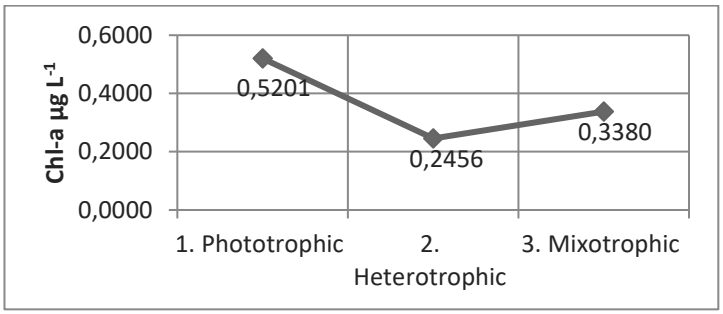

Figure 3. The effect of initial 5 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

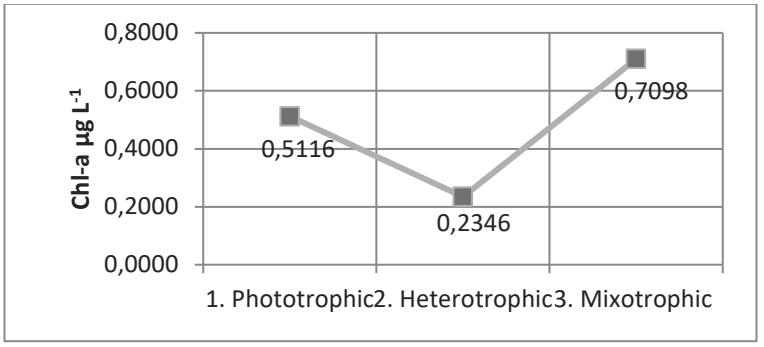

Figure 4. The effect of initial 10 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

Since the chlorophyll concentration of cells is an indicator of photosynthesis, we can say that the decrease in chlorophyll in heterotrophic culture cells is related to the rate of photosynthesis. Due to low chlorophyll production, it can be concluded that the microalgae have preferentially used the available organic carbon as energy and carbon sources in heterotrophic metabolism rather than CO_2 in autotrophic metabolism (Cheirsilp & Torpee, 2012). According to the results of the study, we can say that the photosynthesis and trophic

mode of the algae were transformed into heterotrophic mode as a result of the high carbon source and lack of light in the environment.

Consumption of organic carbon sources by photosynthetic microorganisms' heterotrophic cultivation can decrease chlorophyll content due to changes in photosystem activity (Caporgno et al., 2012). Organic carbon sources cause a decrease in the amount of excitation energy in the photosystem. As a result, a decrease in photosystem activity is observed (Liu et al., 2019). As a result, a decrease in the amount of chlorophyll is observed (Figure 5).

The Analysis Results of Total C.H. Content (mg mL^{-1}) of Biomass

Tables 4-6 show the effects of initial 2.5, 5, and 10 g L^{-1} glucose concentration on the total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. The total C.H. content (mg mL^{-1}) of treated microalgae cells increased significantly with increased glucose concentrations under the heterotrophic (H.C.) and mixotrophic culture (M.C.) conditions. The C.H. contents of cells were affected significantly, especially at 5 and 10 g L^{-1} glucose concentrations.

The analysis determined the mean amount of total C.H. of *Oscillatoria* sp. biomass as 3.5715 mg mL^{-1} under the phototrophic culture (without glucose). Under the mixotrophic culture (presence of light), the experimental results showed that the total C.H. was calculated as 3.6150, 7.9129 and 11.3191 mg mL^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 6-8). Under the heterotrophic culture (absence of light), the results showed that the total C.H. was 4.2969, 8.0990 and 11.5861 mg m L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 9).

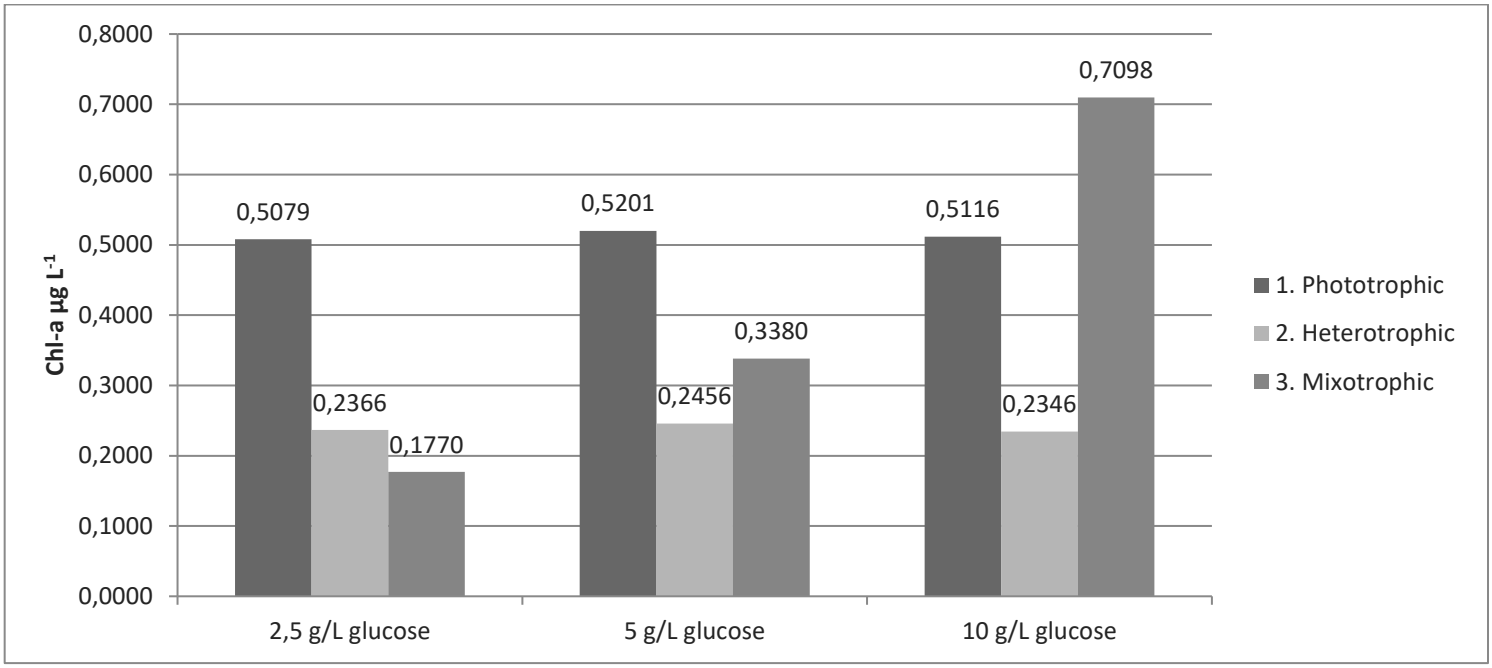

Figure 5. Chlorophyll-a contents of *Oscillatoria* cells grown in different trophic environments and glucose concentrations

Table 4. The effect of initial 2.5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.2927 ±0.002
2. Heterotrophic	4.2969 ±0.002
3. Mixotrophic	3.6150 ±0.001

Table 5. The effect of initial 5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.8836 ±0.001
2. Heterotrophic	8.0990 ±0.003
3. Mixotrophic	7.9129 ±0.001

Table 6. The effect of initial 10 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.5382 ±0.002
2. Heterotrophic	11.5861 ±0.001
3. Mixotrophic	11.3191 ±0.002

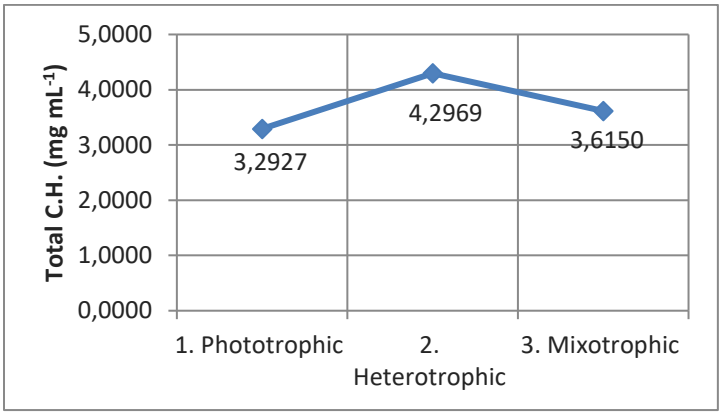

Figure 6. The effect of initial 2.5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

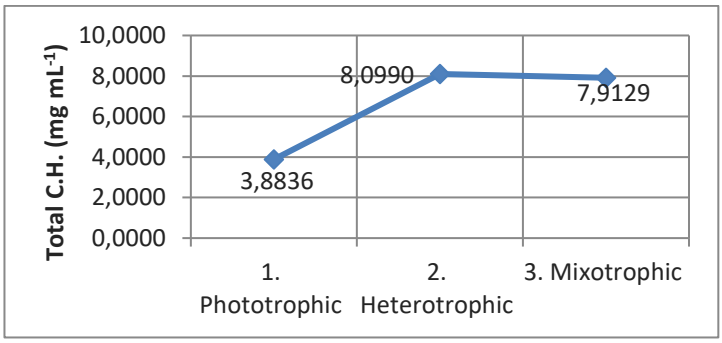

Figure 7. The effect of initial 5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

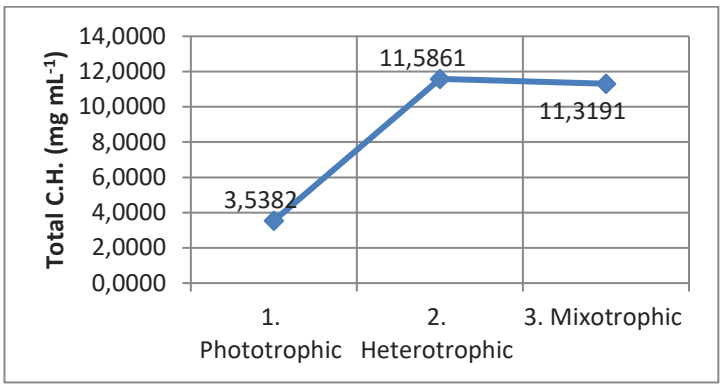

Figure 8. The effect of initial 10 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

Similar to the results of this study, Choi et al. (2019) have determined a high increase in carbohydrates and lipids in *Scenedesmus* cells grown in mixotrophic culture, compared with photoautotrophic and heterotrophic conditions. Glucose, often used as a carbon source for mixotrophic cultivation of various microalgae as it is easy to assimilate, can support *Oscillatoria* cell growth under heterotrophic culture conditions (absence of light). As growth occurs in the presence of glucose, a significant increase in carbohydrate concentration is observed compared to phototrophic control cultures. In this nutrition mode, the microalgae may assimilate different dissolved organic carbon (DOC) sources in addition to the inorganic carbon (CO2) fixed through photosynthesis (Abeliovich & Weisman, 1978; Chojnacka & Marquez-Rocha, 2004; Heredia-Arroya et al., 2010).

The Analysis Results of Growth Estimation (660 -730 nm)

Growth was estimated in each sample by measuring the biomass turbidity of SP homogenised suspension at wavelength 660 nm and 730 nm using a spectrophotometer (Table 7). *Os-*

cillatoria sp. were cultured under controlled laboratory conditions with 16:8 light illumination and aeration. With the cultivation of cells under 25-26 °C, all cultures had an obvious growth except the control group. The recorded optical density of *Oscillatoria* was increased from 0.6798±0.01 at 660 nm and 0.5847±0.01 at 750 nm after 24 hrs to 1.2174±0.002 at 680nm and 1.0243±0.01 at 730nm at the end of 120hrs of the experiment.

Results presented in Figure 10-11 and Table 7 demonstrate the effects of different concentrations of glucose on the biomass growth curves of *Oscillatoria* cells under mixotrophic (M.C) and heterotrophic (H.C) cultivation (120h). The O.D. of microalgae cells increased with the supplement of glucose and the glucose concentration under the H.C. and M.C. conditions. The samples supplied organic carbon source (glucose), especially at 10 g L^{-1} glucose concentrations, displayed superior growth compared with the phototrophic control. In particular, the growth rate of cells grown in M.C. and H.C. cultures was higher than that under phototrophic (control) culture. According to the experimental results, we can say that, compared to photoautotrophic culture, adding carbon sources such as glucose to mixotrophic or heterotrophic environments supports algal growth.

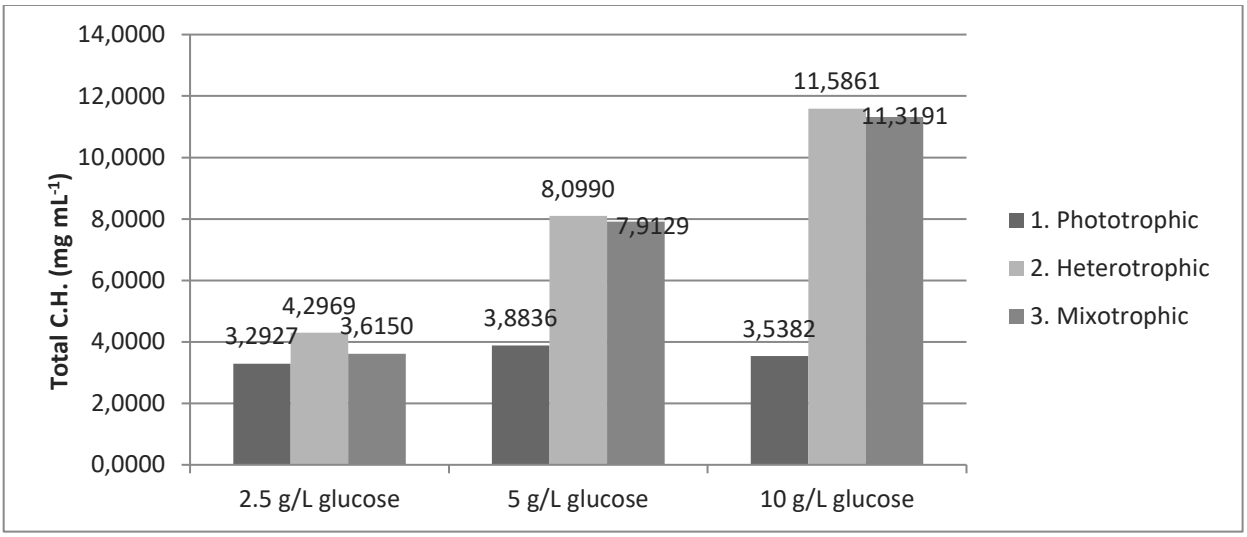

Figure 9. Total C.H. contents of *Oscillatoria* cells grown in different trophic environments and glucose concentrations

Table 7. Mean values of optical density (at 660 and 730 nm) for *Oscillatoria* growth with different concentrations of various glucose supplements at 1 th, 3 th and 5 th days of culture

O.D (660nm)	1 th day	3 th day	5 th day
Control	0.6798	0.6957	0.8412
Mixotrophic culture (2.5 g/L glucose)	0.7488	0.7496	0.7510
Mixotrophic culture (5 g/L glucose)	0.7497	0.7498	0.7563
Mixotrophic culture (10 g/L glucose)	0.8753	0.9586	1.1147
Heterotrophic culture (2.5 g/L glucose)	0.8214	0.8426	0.8515
Heterotrophic culture (5 g/L glucose)	0.8355	0.9551	0.9957
Heterotrophic culture (10 g/L glucose)	0.9597	1.1075	1.2174
O.D (730nm)	1 th day	3 th day	5 th day
Control	0.5847	0.5957	0.6982
Mixotrophic culture (2.5 g/L glucose)	0.6232	0.6863	0.7044
Mixotrophic culture (5 g/L glucose)	0.7411	0.7423	0.7433
Mixotrophic culture (10 g/L glucose)	0.7743	0.8186	1.0177
Heterotrophic culture (2.5 g/L glucose)	0.8022	0.8193	0.8215
Heterotrophic culture (5 g/L glucose)	0.8469	0.8561	0.9025
Heterotrophic culture (10 g/L glucose)	0.8604	0.9757	1.0243

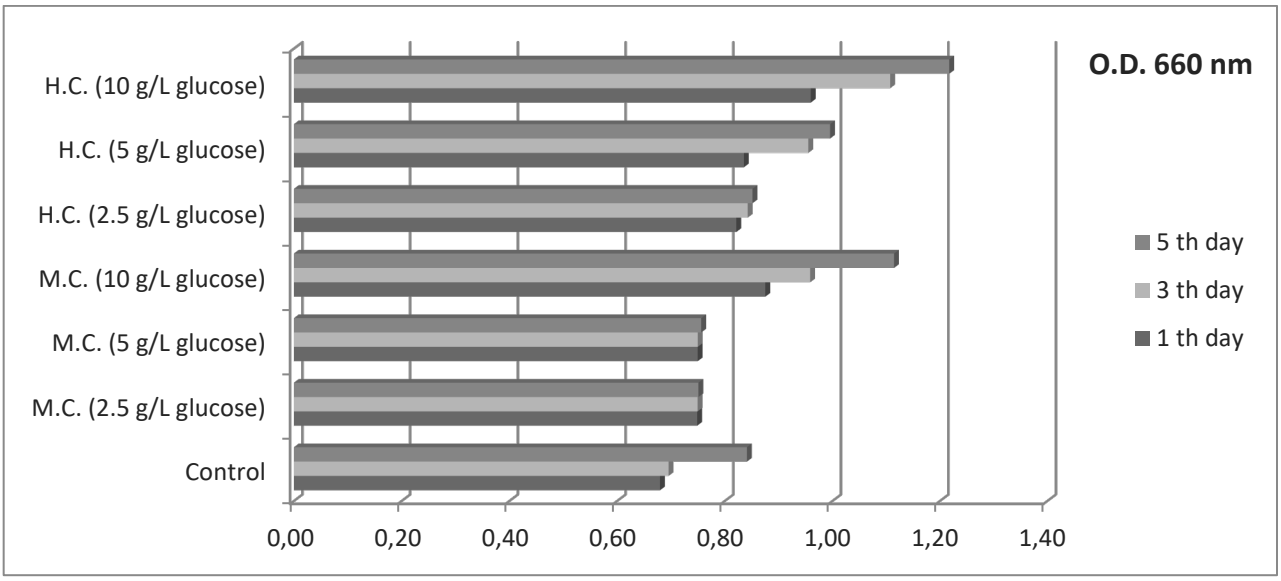

Figure 10. Mean optical density values at 660 nm for *Oscillatoria* growth with different concentrations of various glucose supplement at 1th, 3th and 5th days of culture

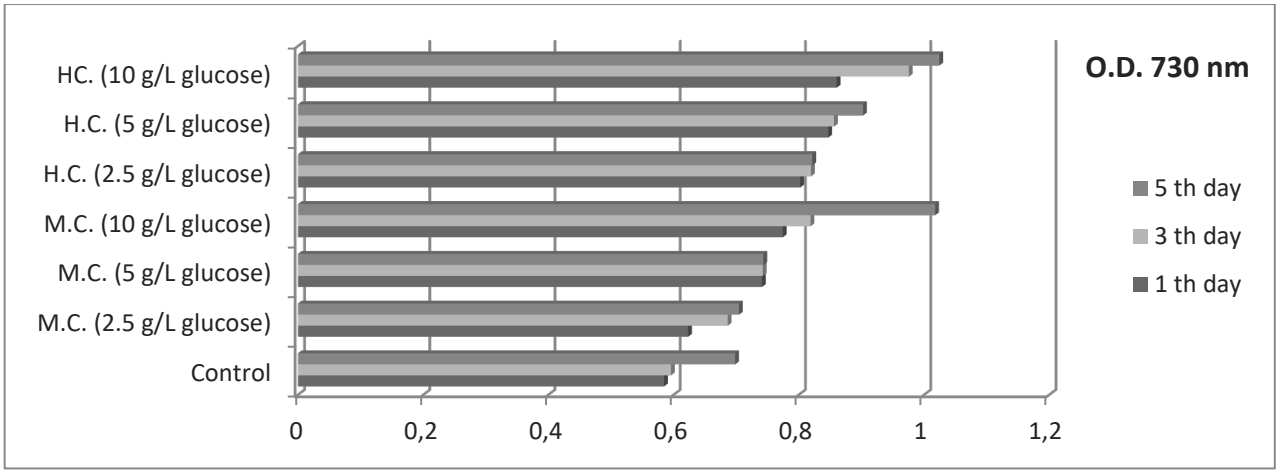

Figure 11. Mean optical density (O.D) values at 730 nm for *Oscillatoria* growth with different concentrations of various glucose supplements at 1 th, 3 th and 5 th days of culture.

Heterotrophic and mixotrophic cultures can present higher growth rates and biomass production than autotrophic ones. In this study results, the heterotrophic biomass productivity of *Oscillatoria* cells in glucose can be 1.74 times higher than the autotrophic one. Besides the reasons that limit biomass production in autotrophic cultivation systems, the bioenergetics of the heterotrophic metabolism can explain the better cultivation performance in dark conditions (Han et al., 2012).

In mixotrophic growth, there are two distinctive processes: photosynthesis (influenced by light intensity) and aerobic respiration (related to the organic substrate concentration). (Ben, 2012). The high cell density of mixotrophic cultures demonstrates that the growth-stimulating effects of light and CO_2 utilisation in mixotrophic cultures were as strong as the effects of glucose (Katarzyna & Andrzej, 2004). Mixotrophic growth offers the opportunity to increase microalgal cell concentration and volumetric productivity greatly. The increase in growth in mixotrophic culture may be because ATP formed in photochemical reactions accelerates anabolism from glucose in mixotrophic culture (Jaemin et al., 2022).

Conclusion

The main aim of the research was to determine the most suitable culture conditions among three different trophic growth environments that would increase the growth rates, chlorophyll-a and total carbohydrate of *Oscillatoria* cells. The experimental results showed that the total C.H. content was increased from 3.5715 to 11.58 61 mg mL^{-1} in the heterotrophic (the absence of light and the presence of 10 g L^{-1} glucose) BG-11 culture conditions. The chlorophyll-a content was increased from 0.1770 µg L^{-1} to 0.7098 µg L^{-1} in the mixotrophic (the presence of glucose and light) BG-11 culture conditions. As a result of the experiment, it was determined that the most suitable culture in terms of total carbohydrate and growth rate was mixotrophic and heterotrophic BG-11 (10 g L^{-1} glucose) culture condition, and in terms of chl-a was mixotrophic culture (10 g L^{-1} glucose). To generalise the results, we can say that microalgae grown in a mixotrophic culture are more effective, especially in increasing carbohydrate and chlorophyll concentrations. The finding suggests that the biomass growth curve, chl-a pigment, and C.H. production of *Oscillatoria* cells could be regulated by controlling the mixotrophic mode especially.

Compliance with Ethical Standards

Conflict of interest: The authors declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

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