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Optimization of Microalgal Biomass Production in Vertical Tubular Photobioreactors

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Abstract: Microalgal biomass is a promising alternative and renewable substrate for bioenergy production. The main problem for its commercial application is to obtain and keep a high level of production by providing microalgae with appropriate conditions for growth. The aim of this study was to determine optimal culture conditions such as temperature, photoperiod, and pH. The amount of biomass by gravimetry, optical density by spectrophotometry, and productivity were analyzed. Suitable values of cultivation parameters allowed for the increased growth and biomass productivity of *Arthrospira platensis* (4.24 $g \cdot L^{-1}$), *Chlamydomonas reinchardtii* (1.19 $g \cdot L^{-1}$), *Chlorella vulgaris* (2.37 $g \cdot L^{-1}$), and *Dunaliella salina* (4.50 $g \cdot L^{-1}$) and optical density for *Ch. reinchardtii* and *C. vulgaris*. These species had maximum biomass productivity of 0.72, 0.12, 0.36, and 0.77 $g \cdot L^{-1} \cdot d^{-1}$, respectively. Productivity was determined by cultivation temperature and for *Ch. reinchardtii* also by pH.

Keywords: microalgae; biomass; optimization; photobioreactor; growth parameters

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1. Introduction

Sustainable and renewable energy sources are an alternative to conventional energy derived from fossil fuels. An important and universal source of renewable energy that allows the production of bioenergy, whether in the form of heat, electricity, or transportation biofuels, is biomass [1]. Bioenergy production increases energy independence [2], and some associated technologies can reduce greenhouse gas emissions [3]. Some biomass sources are products and waste from the agricultural, forestry, municipal, or aquaculture industrial sectors [4]. Since certain biomass types also have non-energy uses and the available feedstock for the bioenergy sector can be limited [5], microalgae have been of interest again. Microalgae are microscopic organisms that have been used in a wide range of industries. Their biomass and cellular components are used in the production of food and supplements, feed, cosmeceuticals, nutraceuticals, and pharmaceuticals [6,7]. Among others, carbohydrates, lipids, and proteins can be obtained from their fast-growing biomass [8]. Biomass and those macromolecules are important for energy production and for biofuels [9–11], including biodiesel [12,13], biohydrogen [14,15], bio-oil [16], or bioethanol [17]. Some microalgal strains can produce liquid hydrocarbons [18] that can be converted into diesel or petrol [10]. The energy potential of algae is wide and the type of fuel produced from them depends on the biomass conversion method (biochemical, thermochemical, or physicochemical). The selection of the appropriate method depends on the amount and type of used biomass, the required form of final energy, and economic efficiency [19]. Biomass is a promising substrate also for biogas production during anaerobic digestion [20]. Compared to other fuels, not only raw biomass but also biomass residues, e.g., after oil pressing, can be used for biogas production [21]. Microalgal biomass for AD does not require drying, which is beneficial for the economic balance of this process [22]. Many authors confirm the significant potential of microalgae for methane production [23–26]. The efficiency of biogas extraction from microalgal biomass depends on the technology (single- or multi-stage processes and dry or wet, mesophilic or thermophilic fermentation)

and the retention time (HRT), but above all on the type of algae, which is related to the chemical composition of their cells. Large amounts of carbohydrates increase the rate of methane fermentation. A higher content of lipids and proteins decreases the rate of biogas production but increases the methane content in the range of 75 to 90% [27]. The integration of bioprocessing pathways makes it possible to obtain not only electricity and different types of fuels, but also value-added chemicals such as lutein or astaxanthin [28].

Production of microalgal biomass on a large scale requires optimization of cultivation technologies [29]. These processes depend on the culture conditions as well as the type of microalgae species. Microalgae are most often grown in an autotrophic method [30] in open pond systems [31] or in photobioreactors [32]. During photosynthesis with light, microalgae convert carbon dioxide into biomass and valuable cellular components [33]. The spectrum of light influences the cell size, amount, and productivity of the biomass [34]. Optimal light intensity values for the growth of different algal species are between 26–400 μmol m^{-2} s^{-1} [35]. Microalgae are grown in an aquatic environment, which must be kept under appropriate conditions of illumination, temperature, pH, and CO_2 concentration [36]. Suitable nutrients are also required for algal growth [37,38].

Under natural conditions, microalgae utilize sunlight, while under laboratory or industrial conditions, artificial lighting is most often used [39]. The intensity of light affects photosynthesis, resulting in biomass yield and its composition [40]. The use of artificial lighting allows to control not only the period of illumination and light intensity but also its spectrum [41]. Special attention is given to light-emitting diodes (LEDs) with their low energy consumption, narrow emission spectrum, long lifetime, and no mercury [42]. Not only the type of light and its intensity are important for the growth and development of microalgae, but also the time of illumination of the culture per day and the meaning photoperiod [43].

Microalgae can grow at temperatures between 5 and 40 °C; however, each species has its own optimal growth temperature. For most microalgae, this is between 20 °C and 30 °C [44]. Temperatures beyond this range can inhibit or completely stop their growth [45]. Temperature significantly affects their metabolism, including nutrient uptake and carbon dioxide fixing efficiency [46]. The production of biomass is inhibited by temperatures that are too low relative to the optimal, while temperatures above the optimum significantly reduce productivity. This can lead to a loss of culture as cell mortality increases [47,48].

For microalgae, the pH is one of the most critical environmental conditions [49]. They usually grow in a pH from 6 to 9 [37], despite the fact that many of them can tolerate wide pH ranges [50]. The pH increases during the light period and decreases in darkness due to respiration [51]. Furthermore, the pH changes can affect enzymes [52], the functions of cell membranes, and photosynthesis [53] since the pH of the culture medium affects the availability of CO_2 [46].

Optimization of microalgae growth conditions is crucial for the intensification of biomass production for energy purposes, including biofuel production. The suitable abiotic conditions for this process should be appropriate to the type of photobioreactor. The aim of this study was to determine the optimal cultivation parameters affecting the growth of four selected microalgae species in vertical tubular photobioreactors. Photobioreactors with capacities similar to those used in this study represent the first stage of scale-up production. The effects of temperature, lighting time, and the pH of the growth medium were analyzed. All of the parameters were evaluated at the same time, and the results presented represent the interaction between those factors. We hypothesize that an appropriate configuration of optimal values for each parameter will positively affect the production of microalgal biomass. The aim of the optimization is to increase the amount of biomass as feedstock for anaerobic digestion.

2. Materials and Methods

2.1. Microalgae Strains

The presented study is a continuation of previously carried out experiments [54] on the production of microalgal biomass as a substrate for anaerobic digestion. In the preliminary study, 16 strains of microalgae were analyzed, and for the next stages, based on the results of biomass production efficiency, four of them were selected. The following species were used in this study: *Chlorella vulgaris* (BA 002) from the Culture Collection of Baltic Algae (CCBA, University of Gdansk, Gdansk, Poland) and *Chlamydomonas reinhardtii* (CHL 152), *Arthrospira platensis* (CYA 428), and *Dunaliella salina* from the culture collection of the Department of Renewable Energy Engineering at the West Pomeranian University of Technology in Szczecin, Poland.

2.2. Preincubation Conditions

Microalgae were kept in F/2 synthetic culture medium [55] with a composition of ($g \cdot L^{-1}$): $NaNO_3$–75, $NaH_2PO_4 \cdot 2H_2O$–5, microelement solution (1 $mL \cdot L^{-1}$): Na_2EDTA–4,16, $FeCl_3 \cdot 6H_2O$–3,15, $CuSO_4 \cdot 5H_2O$–0.01, $ZnSO_4 \cdot 7H_2O$–0.022, $CoCl_2 \cdot 6H_2O$–10, $MnCl_2 \cdot 4H_2O$ 0.18 and $NaMoO_4 \cdot 2H_2O$–0.0063, vitamins solution (1 $mL \cdot L^{-1}$): cobalamin (vitamin B12)–0.01, thiamine (vitamin B1)–0.1, and biotin (vitamin H)–0.005. For the *D. salina* and *A. platensis* species, the growth medium was enriched by sodium chloride (NaCl) to a salinity of 8‰. In addition, 1 $g \cdot L^{-1}$ of sodium bicarbonate ($NaHCO_3$) was added to *A. platensis* medium. Inoculum was prepared in 1 L photobioreactors, which were filled with 700 mL of sterile F/2 culture medium adjusted to pH 7, followed by the addition of 70 mL culture of each microalgae. These cultures were kept for 7 days at 23 ± 1 °C using sodium light (High Pressure Sodium, HPS; PHILIPS, Amsterdam, The Netherlands) at 125 µmol m^{-2} s^{-1} and a photoperiod of 12/12 h (light/dark cycle). The photobioreactors were aerated with compressed air with an atmospheric concentration of carbon dioxide using a membrane pump at a flow rate of 10 $L \cdot min^{-1}$.

2.3. Experimental Setup

The microalgae were grown in vertical tubular photobioreactors (Aqua Medic, Bissendorf, Germany) with a capacity of 2.5 L (Figure 1). They were filled into 2 L of sterile F/2 culture medium and 200 mL of inoculum. The LED lighting (HOLDBOX, Żabia Wola, Poland) with white, red (wavelength from 600 to 700 nm), and blue (wavelength from 400 to 500 nm) diodes was used in the experiment. The light intensity was 130 µmol m^{-2} s^{-1}. The carbon dioxide required for photosynthesis came from the compressed atmospheric air supplied to the photobioreactors using an Aqua Medic Mistral 2000 pump with a flow of 0.9 $m^3 \cdot h^{-1}$. This also allowed for the proper stirring of the culture medium and homogeneous conditions throughout the volume of the photobioreactor. A sterile 0.22 µm polytetrafluoroethylene (PTFE) filter was used for air filtration. The photobioreactors had air-filter-protected valves to remove the oxygen produced during photosynthesis. This experiment was carried out as a batch culture for 15 days. Optimization was conducted for photoperiods of (12/12, 18/6, and 24/0 h in the light/dark cycle), temperatures of (20, 25, and 30 °C), and for pH values of (7, 8, 9). The microalgae cultivation parameters are presented in Table 1.

Table 1. Microalgal biomass production parameters.

Photobioreactor Capacity [L]	CO_2 Dose	Light Intensity [µmol m^{-2} s^{-1}]	Temperature [°C]	Photoperiod [h; Light/Dark]	pH
2.5	atmospheric concentration	130	20	12/12	7
			25	16/8	8
			30	24/0	9

Figure 1. Microalgae cultivation system.

2.4. Analytical Methods

Optimal conditions for microalgae cultivation were determined by measuring the amount of biomass and optical density (OD) in the culture.

The dry weight of the microalgae was determined by the gravimetric method. Their biomass was analyzed using a moisture analyzer (AXIS ATS60, Gdańsk, Poland). Each day, 30 mL samples were taken from particular photobioreactors and centrifuged (Eppendorf, Hamburg, Germany) at 4000× g for 20 min. After centrifugation, the biomass was transferred to aluminum dishes and dried at 105 °C until a constant mass was obtained. After this, the dishes with the biomass were cooled in the desiccators and then weighted. The amount of biomass was determined on the first day of culture start and every 24 h for the next 15 days. The results were given in g·L^{-1}. Productivity (g·L·d^{-1}) was determined based on the biomass yield using the following formula:

$$Biomass\ productivity\ (BP) = \frac{B_f - B_0}{\mathrm{d}} \quad (1)$$

where

B_f is the final amount of biomass (g), B_0 is the initial amount of biomass (g), and d is the cultivation time (day).

2.5. Statistical Analysis

All measurements were carried out in triplicate and the data were expressed as mean value ± standard deviation (SD). The results were analyzed using a computer program (Statistica version 13.3; Dell Inc., Tulsa, OK, USA). Analysis of variance and a post-hoc Tukey's tests were performed at statistical significance of $p \leq 0.05$.

3. Results and Discussion

3.1. Microalgal Biomass Concentration and Productivity

The effectiveness of microalgal biomass production was dependent on the time of culture lighting, temperature, and the pH of the culture medium, and it varied between strains (Figure 2). Under the same cultivation conditions, the highest values were observed for *A. platensis* and *D. salina*. For *A. platensis*, the amount of biomass ranged from 3.08 g·L^{-1}

to 4.24 g·L^{-1}. The maximum value was found at 20 °C, 12/12 h in the light/dark, cycle and at pH 8. According to Li et al. [56], most *Arthrospira* strains prefer an alkaline pH of around 9.0–9.5; however, in this study, the optimum pH was lower. A range of variables determines the growth of microalgae. It is possible that in an optimal combination of temperature and photoperiod, maximum biomass values can be observed at lower pH values. Chaiklahan et al. [57] noted 1.42 ± 0.11 g·L^{-1} for a photoperiod of 24/0 h and a PAR light intensity of 2300 µmol m^{-2} s^{-1}. The efficiency of microalgal biomass production can be increased by optimizing environmental factors or the composition of the culture medium. If the biomass is destined for energy purposes, then the costs associated with nutrient supply are important. Madkour et al. [58] substituted all of the components of Zarrouk's growing medium with lower cost and commonly available fertilizers and chemicals. The authors obtained a maximum biomass yield of 0.813 ± 0.018 mg·L^{-1} with 0.840 ± 0.008 mg·L^{-1} when the microalgae were grown on an optimal medium. De Castro et al. [59] in a study on the effect of sodium bicarbonate and sodium nitrate on the biomass production of this strain obtained 3270 mg·L^{-1}. Based on these results, it can be concluded that, for *A. platensis*, modification of the abiotic factors allows for more favorable results.

The use of microalgae in commercial applications requires a large amount of biomass, but the yield of *Ch. reinchardtii* in this study was rather low, ranging from 0.90–1.19 g·L^{-1}. Lower temperatures resulted in less suitable conditions for the growth and development of these microalgae. The average biomass at 20 °C was 1.01 g·L^{-1}, while at 30 °C, it was 1.12 g·L^{-1}. The optimum pH for this strain was 7–8, while the photoperiod was 18/6 h (light/dark). Vargas et al. [60] also obtained 1073.33 ± 23.09 g·L^{-1} of biomass, although under different conditions (32 °C, pH 6.2). Abd El Baky and El Baroty [61] carried out a larger scale study (400 L photobioreactor) and, under limited availability of N, P, and S and with 6% CO_2 introduced into the culture, obtained a biomass yield of as much as 3.11 g·L^{-1}. This suggests how important suitable carbon dioxide levels could be for some microalgal strains [62].

The average amount of *C. vulgaris* biomass in particular photobioreactors ranged from 1.02 g·L^{-1} to 2.37 g·L^{-1}. Biomass decrease was observed at extreme temperatures, as well as at extreme pH values. The optimal conditions for *C. vulgaris* cultivation, considering all of the analyzed parameters, were a temperature of 25 °C, a photoperiod of 18/6 h in the light/dark cycle, and a pH of 8. A suitable pH provides an optimum ratio of HCO_3^- and CO_2 for carbon assimilation [63]. In a previous study [64], biomass production was optimized for the type of light used in cultivation (high-pressure sodium light or light-emitting diode), photoperiod, and pH, and a maximum of 546 ± 7.88 mg·L^{-1} dry weight of biomass was obtained, which confirms that temperature is an important factor determining the growth of microalgae and the increase in biomass production.

The average biomass content for *D. salina* in the present study, where only the combinations of selected cultivation parameters were used, ranged from 3.36 g·L^{-1} to 4.50 g·L^{-1}. It was noted that the amount of biomass decreased with the increasing temperature. Similar changes were observed when the lighting time of the culture increased. As indicated by Xu et al. [65], intracellular starch content increases during periods of light, while it decreases in the dark. This may have significance for energy production from microalgal biomass. According to the maximum values observed in the presented study, beneficial conditions for *D. salina* cultivation are 20 °C, pH 8, and a photoperiod of 12/12 h (light/dark), although the optimal temperature may change depending on the cultivation parameters [66]. Gastelum-Franco et al. [67] kept cultures of *D. salina* in F/2 medium at a temperature of 24 ± 2 °C, a pH 8.2, and for a 12/12 h photoperiod and obtained 2.93 ± 0.40 g·L^{-1}. Statistical analysis of the presented results did not confirm significant changes in biomass productivity as an interaction between temperature and pH. Several studies have been carried out to increase the biomass productivity of *D. salina* by modifying the composition of culture media or by modifying culture conditions [68]. Morowvat and Ghasemi [69] optimized the composition of the culture medium for glucose content and nitrate and phosphate concentration and obtained a biomass concentration of 1.015 g·L^{-1}. Ahmed et al. [70] optimized biomass

production by changing the salinity level and, after 21 days of culture, obtained at 2 M NaCl 1231.66 ± 1. 26 mg·L^{-1}. In this study, the biomass yields were higher than those presented by other authors, indicating that optimizing the environmental parameters for *D. salina* had a more beneficial effect than modifying the composition of the culture medium.

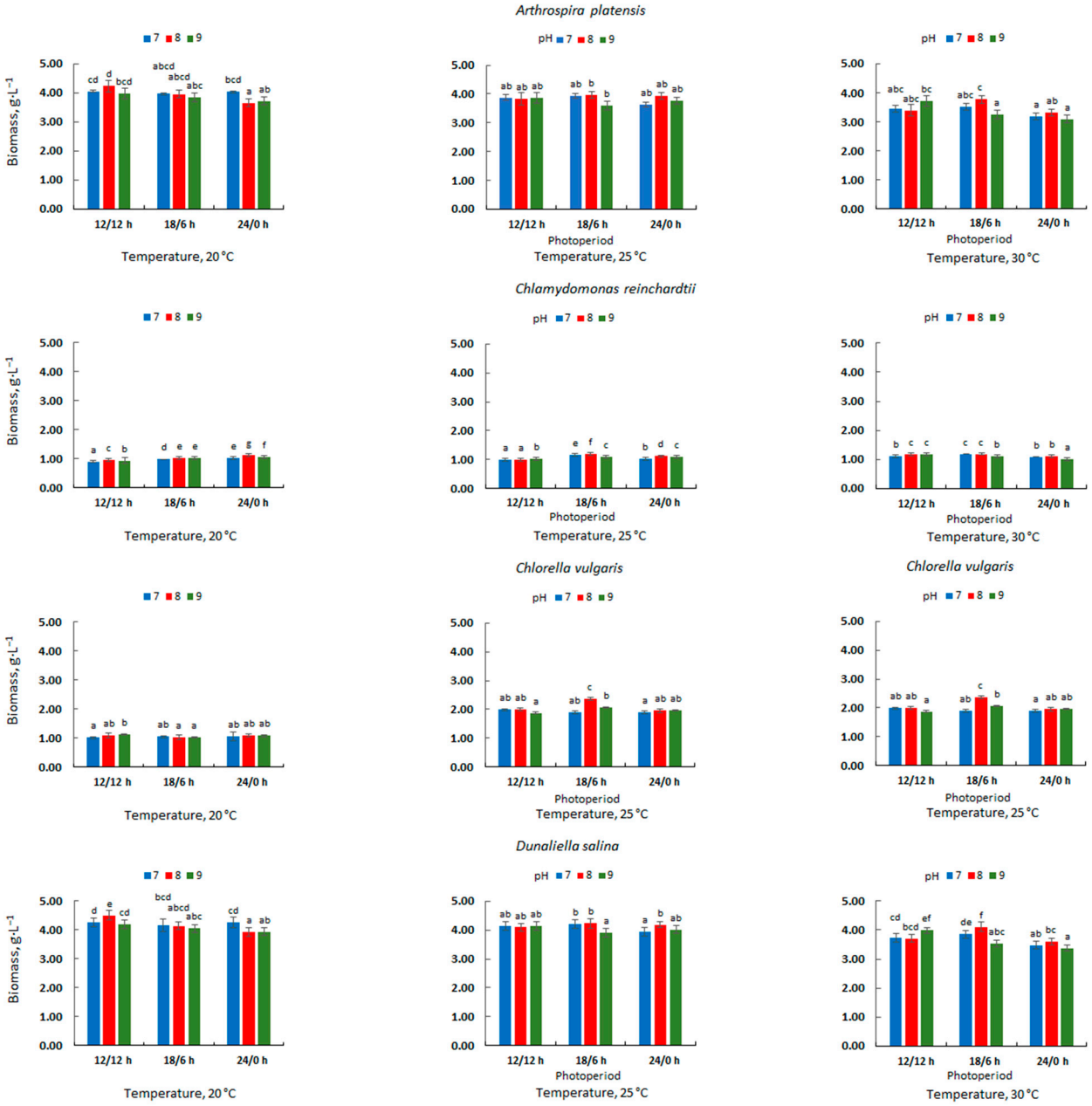

Figure 2. The average amount of microalgal biomass during different cultivation conditions. Different letters above the error bars (i.e., ±SD) indicate significant differences ($p < 0.05$) between the means; means followed by the same letter do not statistically differ from each other (Tukey's post-hoc test).

In different types of photobioreactors, the optimum growth conditions for the same strains may differ (Table 2). Additionally, a change in the scale of biomass production, with varying volumes, alters cultivation conditions. Optimizing cell culture conditions is essential to improve microalgae's industrial productivity [71].

Table 2. Comparison of microalgal biomass production during different cultivation conditions.

Microalgae	Cultivation Conditions			Amount of Biomass [g·L^{-1}]	Photobioreactor Type/Capacity [L]	References
	Temperature [°C]	Photoperiod [h/h Light/Dark]	pH			
A. platensis	20	12/12	8	4.24	Tubular: 2.5	This study
	Not controlled	13/11	9–10	0.93	Bubble 10.1	[72]
	Room temp.	24/0	9	1.50	Bubble: 2	[73]
	30	24/0	9.6	3.06	Airlift: 2.5	[74]
Ch. reinchardtii	30	18/6	7–8	1.19	Tubular; 2.5	This study
	24–32	n.r [1]	n.r	0.5–0.7	Flask; 0.25	[75]
	32	24/0	6.2	1.07	Flask; 0.5	[60]
	25	14/10	n.r.	1.74	Flat plate; 1.5	[76]
C. vulgaris	25	18/6	8	2.37	Tubular; 2.5	This study
	25	n.r	7	0.824	Baffled; 5	[63]
	20–25	20/4	4–7	0.317	Airlift; 6.6	[77]
	25	24/0	9	0.150–0.205	Flask; 0.25	[78]
D. salina	20	12/12	8	4.50	Tubular; 2.5	This study
	25	n.r	n.r	2.13	Bubble; 0.25	[79]
	20	18/6	n.r	0.801	Bubble; 2	[80]
	22	16/8	7.7	0.025	Flask; 0.25	[81]

[1] n.r–not reported.

The productivity of microalgal biomass is presented in Table 3. The average values varied according to the temperature, time of culture lighting, and pH. For *A. platensis*, productivity ranged from 0.49 g·L^{-1}·d^{-1} to 0.72 g·L^{-1}·d^{-1}. Similar values (maximum 0.62 ± 0.05 g·L^{-1}·d^{-1}) were reported by Chaiklahan et al. [57]. In the present study, high productivity was promoted by cultivation under controlled conditions in photobioreactors. Guidi et al. [82] cultivated this strain under a greenhouse in an 8000 L raceway and determined biomass productivity of 0.08 g·L^{-1}·d^{-1}.

Table 3. Comparison of microalgal biomass productivity.

Temperature [°C]	Photoperiod [Light/Dark]	pH	Biomass Productivity [g·L^{-1}·d^{-1}]			
			Arthrospira platensis	*Chlamydomonas reinchardtii*	*Chlorella vulgaris*	*Dunaliella salina*
20	12/12	7	0.69 ± 0.03	0.06 ± 0.01	0.10 ± 0.00	0.77 ± 0.03
		8	0.72 ± 0.04	0.07 ± 0.00	0.11 ± 0.01	0.81 ± 0.04
		9	0.68 ± 0.03	0.06 ± 0.01	0.12 ± 0.03	0.74 ± 0.03
	18/6	7	0.66 ± 0.03	0.08± 0.01	0.29 ± 0.02	0.72 ± 0.03
		8	0.63 ± 0.03	0.08 ± 0.01	0.30 ± 0.02	0.69 ± 0.02
		9	0.66 ± 0.03	0.08 ± 0.01	0.26 ± 0.01	0.71 ± 0.03
	24/0	7	0.50 ± 0.03	0.10 ± 0.02	0.20 ± 0.00	0.59 ± 0.03
		8	0.50 ± 0.02	0.11± 0.01	0.19 ± 0.00	0.60 ± 0.03
		9	0.53 ± 0.02	0.11 ± 0.01	0.20 ± 0.00	0.64 ± 0.02
25	12/12	7	0.67 ± 0.03	0.08 ± 0.01	0.10 ± 0.01	0.75 ± 0.04
		8	0.66 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.71 ± 0.03
		9	0.65 ± 0.03	0.08 ± 0.01	0.10 ± 0.01	0.72 ± 0.03
	18/6	7	0.65 ± 0.02	0.11 ± 0.01	0.27 ± 0.01	0.71 ± 0.03
		8	0.65 ± 0.02	0.12 ± 0.01	0.36 ± 0.01	0.71 ± 0.03
		9	0.59 ± 0.02	0.10 ± 0.01	0.31 ± 0.01	0.65 ± 0.02
	24/0	7	0.51 ± 0.02	0.12 ± 0.02	0.19 ± 0.00	0.61 ± 0.03
		8	0.56 ± 0.03	0.12 ± 0.01	0.20 ± 0.00	0.67 ± 0.03
		9	0.49 ± 0.03	0.10 ± 0.01	0.20 ± 0.00	0.58 ± 0.04
30	12/12	7	0.69 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.77 ± 0.03
		8	0.62 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.69 ± 0.03
		9	0.64 ± 0.03	0.09 ± 0.00	0.11 ± 0.00	0.71 ± 0.03
	18/6	7	0.60 ± 0.02	0.08 ± 0.01	0.27 ± 0.01	0.65 ± 0.02
		8	0.63 ± 0.03	0.10 ± 0.01	0.28 ± 0.01	0.68 ± 0.03
		9	0.61 ± 0.02	0.10 ± 0.01	0.29 ± 0.01	0.65 ± 0.02
	24/0	7	0.47 ± 0.02	0.10 ± 0.01	0.20 ± 0.01	0.56 ± 0.02
		8	0.49 ± 0.03	0.10 ± 0.01	0.19 ± 0.00	0.59 ± 0.03
		9	0.47 ± 0.02	0.08 ± 0.01	0.20 ± 0.01	0.56 ± 0.02

Significantly lower values were observed for *Ch. reinchardtii*, with a maximum of 0.12 $g \cdot L^{-1} \cdot d^{-1}$ at 25 °C for a photoperiod of 18/6 h in the light/dark cycle and pH 8 and for a photoperiod of 24/0 and a pH range of 7–8. By optimizing the physicochemical parameters (pH 6.5–7.0 and 30 °C), Banerjee et al. [83] improved biomass productivity to 512 $mg \cdot L^{-1} \cdot d^{-1}$. Research by Kong et al. [84] indicates that the optimal pH for *C. reinhardtii* is 7.5. Under such conditions in Biocoil photobioreactors, these authors obtained 2.00 ± 0.03 $g \cdot L^{-1} \cdot d^{-1}$.

In this study, the biomass productivity of *C. vulgaris* was at a relatively low level. A maximum value of 0.36 $g \cdot L^{-1} \cdot d^{-1}$ was observed at 25 °C for a photoperiod of 18/6 h (light/dark) and pH 8. Kim et al. [71] reported increases in the productivity of the *Chlorella* sp. genus from a level of 0.28 $g \cdot L^{-1} \cdot d^{-1}$ to 0.51 $g \cdot L^{-1} \cdot d^{-1}$ in mixotrophic cultivation with glucose. Anyanwu et al. [85] analyzed the effects of introducing dark periods and different types of LEDs to lighting photobioreactors (red, white, and green) and without a dark period, and using white light, they obtained significantly higher biomass productivity of 2.438 $g \cdot L^{-1} \cdot d^{-1}$. A suitable light type and light intensity and an appropriate photoperiod affect the chemical composition of cells [86] which is important if microalgae are used as a substrate for bioenergy production.

The productivity for *D. salina* was high and ranged from 0.56–0.81 $g \cdot L^{-1} \cdot d^{-1}$. Values decreased with increasing culture lighting time, while the effect of pH was unclear. There were no statistically significant differences in biomass productivity at different pH values. Abarna et al. [87] analyzed the enhancement of biomass productivity through the application of liquid seaweed fertilizers in cultivation and obtained a maximum of 0.038 $g \cdot L^{-1} \cdot d^{-1}$. The evaluation of biomass productivity was the first stage of the analyses of *D. salina*'s commercial suitability carried out by Borovkov et al. [88]. These authors cultivated *D salina* for 14 days. In the mid-incubation time, they reduced the temperature from 27–28 °C to 23–24 °C and increased the average intensity of light from 40 $W \cdot m^{-2}$ to 80 $W \cdot m^{-2}$ and obtained for one of the three tested strains 0.18 $g \cdot L^{-1} \cdot d^{-1}$.

The optimum growth temperatures for the different microalgal species ranged from 20 to 30 °C. Setting an optimum value for this parameter avoids cellular stress [89] and increases biomass production. Our studies indicate that this parameter is related to other environmental factors. The light/dark cycle influences the rate of photosynthesis, so extending the light phase can significantly increase biomass yield [90]. Such a correlation was not observed in this study, suggesting that the optimum for this parameter depends on the microalgal species. This has also been confirmed by studies carried out by other authors [91,92]. An important parameter affecting microalgal growth, where the optimum and tolerance also depend on the microalgal species [46], is pH. This factor influences the solubility of inorganic carbon in culture [93] and can significantly modify the physicochemical properties of the cells [94], so it is crucial to determine and keep optimal values during cultivation. Most of the microalgae tested preferred slightly alkaline conditions, even *A. platensis*. In this strain, this consideration may have resulted from interaction between the applied factors.

3.2. Optical Density in Microalgal Culture

The average OD_{680} values determined in the study are presented in Figure 3. For *A. platensis*, a significant effect was determined only for the temperature and photoperiod; the effect of the pH of the culture medium and the interaction between the factors studied were not significant. At 20 °C, the highest OD_{680} value (0.421) was recorded for the 24/0 h photoperiod in the light/dark cycle. At 25 °C, the OD_{680} increased to 0.509 for the 12/12 h photoperiod, while at 35 °C a value of 0.455 was recorded for the 18/6 h light/dark cycle. Hussin et al. [73] analyzed the effect of aeration rate, pH, and light intensity for two morphological forms of *A. platensis* and obtained a cell density (OD_{680}) of 1.287 ± 0.019 for helical forms and 1.318 ± 0.059 for straight forms. Due to the difference in light dispersion during the measurement associated with different cell sizes according to Shagerl et al. [95], OD values cannot be used to compare different species.

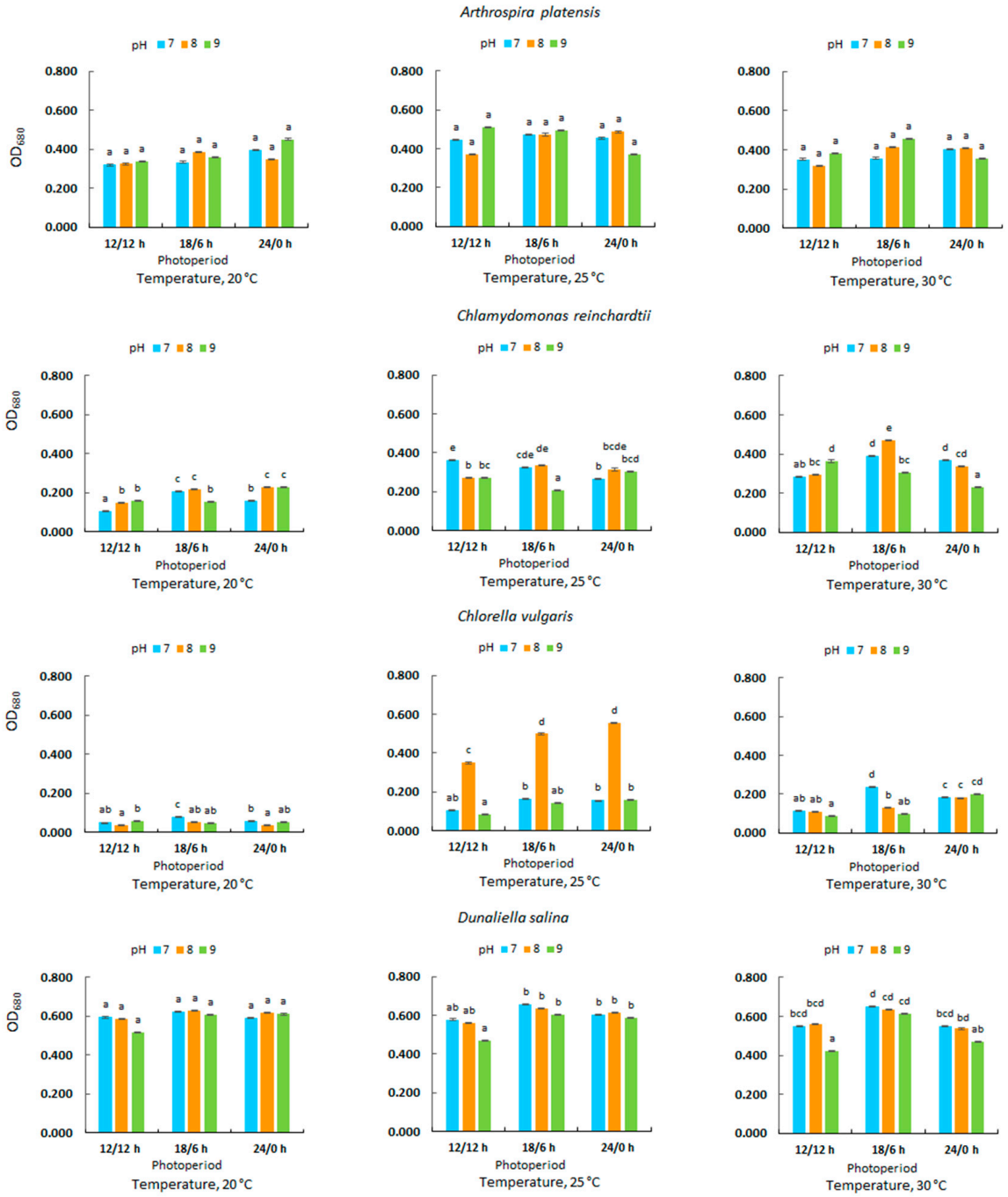

Figure 3. The average optical density during different cultivation conditions. Different letters above the error bars (i.e., ±SD) indicate significant differences ($p < 0.05$) between the means; means followed by the same letter do not statistically differ from each other (Tukey's post-hoc test).

For *Ch. reinchardtii*, all analyzed of the factors and their interactions had a significant effect on optical density. The highest OD680 was recorded at 30 °C for 18/6 h (light/dark cycle) and pH 8 (0.468). Ivanov et al. [96] cultivated *Ch. reinchardtii* for 6 days at 30 °C, and then, after dilution of the growth medium, they set up two combinations and continued incubation at 30 °C and 39 °C. These authors observed advantageous changes in optical density at 30 °C.

Additionally for the *C. vulgaris* strain there was a significant interaction effect of the analyzed growth parameters. The highest values for optical density were observed at the temperature of 25 °C and for pH 8 and ranged from 0.349 for the 12/12 h photoperiod to

0.557 for 24/0 h of continuous lighting. Sharma et al. [97] analyzed the effect of light type (fluorescent or natural) and temperature in two ranges, 25–30 °C and 30–35 °C, and the maximum OD_{670} value for this microalgae species (0.42) was observed during cultivation under natural light at lower temperatures.

For *D. salina*, there was no significant interaction effect between the temperature, the pH of the growth medium, and the photoperiod. At 20 °C, the OD_{680} was 0.630 for the 18/6 h photoperiod and pH 8, while at 25 °C and 35 °C, it was 0.659 and 0.650, respectively, for 18/6 h lighting and for pH 7. The absence of cell walls in this strain is an unquestionable advantage [70]. It simplifies the pretreatment of such substrates and the hydrolysis of components accumulated in the microalgal cells.

The amount of biomass was correlated with the optical density for all of the microalgae; however, the correlation coefficient had a relatively low value between r = 0.35 for *D. salina* to r = 0.62 for *C. vulgaris*. Determination of optimal growing conditions is one of the most important steps in starting large-scale biomass production. Direct weight measurement is a reliable indicator of biomass growth efficiency; however, it is a long-term and time-consuming procedure. In many studies, spectrophotometric measurements of optical density are carried out as an alternative, although the use of this parameter is not evident. Earlier studies [98] on *C. vulgaris* confirmed the correlation between gravimetric and spectrophotometric measurements, but optical density is based on cellular pigment, and its content varies with culture age and culture conditions, which can lead to incorrect results [99]. Algal cell size varies within the growth cycle [100] and can increase with the increasing temperature of cultivation [101]. Thus, OD measurements can be used for online monitoring of biomass production [102] and as a complement to gravimetric methods [95].

4. Conclusions

Microalgae are a promising renewable source for bioenergy production. Biomass and components accumulated in the microalgal cells can be converted to heat, electricity, and different types of biofuels. A simple and low-cost method to convert microalgal biomass into a sustainable renewable energy source is anaerobic digestion. This process allows for the utilization of wet biomass, which has a positive effect on the balance of energy obtained compared with that invested, including for biomass production and harvesting. Production of large amounts of biomass requires optimization of cultivation conditions. There are many factors that affect microalgae growth. Besides nutrient availability, critical environmental parameters for cultivation include light, temperature, and pH. Providing optimal growth conditions increases biomass yield and productivity. Therefore, microalgae were cultured under different temperatures, pH values, and light/dark times over a 24-h cycle. The results show that the interaction between these essential abiotic factors for cell growth had a positive effect on *A. platensis* and *D. salina* and resulted in over 4 $g \cdot L^{-1}$ of dry weight for both strains. For the other strains, it is necessary to extend the study to other environmental factors or to modify the growth media to obtain more positive results. For all of the tested species, the most important determinant of biomass productivity was temperature.

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