

## Stimulatory effect of indole-3-acetic acid and continuous illumination on the growth of *Parachlorella kessleri*\*\*

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**A b s t r a c t.** The effects of the phytohormone indole-3-acetic acid and various conditions of illumination on the growth of *Parachlorella kessleri* were investigated. Two variants of illumination: continuous and photoperiod 16/8 h (light/dark) and two concentrations of the phytohormone –  $10^{-4}$  M and  $10^{-5}$  M of indole-3-acetic acid were used in the experiment. The results of this study show that the addition of the higher concentration of indole-3-acetic acid stimulated the growth of *P. kessleri* more efficiently than the addition of the lower concentration of indole-3-acetic acid. This dependence can be observed in both variants of illumination. Increased biomass productivity was observed in the photoperiod conditions. Both the addition of the phytohormone and the conditions of the illumination had an impact on the number of *P. kessleri* cells. An increased number of cells was observed under the conditions of continuous illumination. This result has shown that the continuous illumination and the higher concentration of the phytohormone stimulated the growth of *P. kessleri* more effectively than the shorter duration of light (16/8 h (light/dark)).

**K e y w o r d s:** light, photoperiod, microalgae, indole-3-acetic acid, *Parachlorella kessleri*

### INTRODUCTION

In recent years, there has been growing interest in the biomass of unicellular algae as an alternative energy source. Microalgae, specifically those included in the group of green algae, can be used in production of the third generation of biofuels: biodiesel, biohydrogen, and biomethanol. Algal biomass can also be used in nutrition as food supplements, which are a source of many valuable substances as well as micro- and macronutrients, in the pharmaceutical industry as nutraceuticals, and in cosmetic industry as cosmeceuticals (Mata *et al.*, 2010).

Despite such wide possibilities of using algal biomass, commercialization of biomass production is still a challenge due to the high costs. An increase in the efficiency of production of biomass and valuable intracellular metabolites can improve the profitability of algal cultivation. Therefore, it is important to understand better the factors influencing the growth of microalgae. The main factors that exert an effect on the growth of algae include light, access to nutrients, temperature, pH, salinity, environmental stress, as well as addition of other growth-promoting substances, such as plant hormones (Markou *et al.*, 2013; Salama *et al.*, 2014).

Plant hormones, also called phytohormones, are signal molecules that induce a reaction in the plant organism already at trace concentrations. The main phytohormones *e.g.* gibberellins, auxins, cytokinins (CKs), abscisic acid, ethylene, and brassinosteroids (BRs) are plant growth and development regulators. The most common and best explored auxin is indole-3-acetic acid (IAA) (Lu and Xu, 2015).

Phytohormones are also found in algae at comparable concentrations as those in terrestrial plants. The greatest number of plant hormones has been detected in *Chlorophyta* (green algae). IAA has been detected in several unicellular and multicellular algal species, *i.e.* *Chlorella*, *Enteromorpha*, *Cladophora*, *Caulerpa* (Chlorophyta) *Fucus*, *Laminaria*, *Porphyra*, *Botryocladia* (Rhodophyta), *Ascophyllum*, *Macrocystis* (Phaeophyta), and several others. The spectrum of biological activities of algal hormones

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partly overlaps with that in terrestrial plants; however, it is being discussed whether the function of the algal hormonal system is the same as in terrestrial plants (Lau *et al.*, 2009).

There are reports in the literature that the addition of phytohormones, particularly auxins, to microalgal cultures can increase the productivity of biomass as well as the number of cells and the content of metabolites in microalgal biomass. Exogenous auxin increases the growth rate of algae such as *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Haematococcus pluvialis*, *Pleurochrysis carterae*, and *Phaeodactylum tricoratum* (Lu and Xu, 2015; Park *et al.*, 2014; Piotrowska-Niczyporuk and Bajguz, 2014).

Light is one of the key regulators of the cell cycle and interacts with plant hormones. Both light intensity and the cycle of alternating consecutive periods of light and darkness, i.e. photoperiod, are important. The quantity and quality of light is reflected in the amount of energy available for photosynthesis. Alternating cycles of light and darkness reflect natural environmental conditions of algal growth (Krzemińska *et al.*, 2014, 2015; Strik *et al.*, 2014).

*Parachlorella kessleri* (*Chlorophyceae*) is a unicellular freshwater green alga classified in the class *Trebouxiophyceae*. *P. kessleri* is characterized by a high growth rate and high biomass, starch, and lipid productivity. Moreover, it exhibits good tolerance to high temperature and a low tendency to form aggregates (Fernandes *et al.*, 2013). These traits indicate that the species can be used in industrial productions of biomass.

The aim of the study was to determine the cumulative effect of addition of indole-3-acetic acid (IAA) and two light regimes: 16/8 h (light/dark) and continuous illumination on the growth parameters of *Parachlorella kessleri*.

#### MATERIALS AND METHODS

*Parachlorella kessleri* was obtained from the Culture Collection of Autotrophic Organisms (CCALA) from Dukelská in Czech Republic. The axenic cultures of *P. kessleri* were cultured in Bold Basal medium. The experiments were done in 300 ml sterile Erlenmeyer flasks with 150 ml of the medium with orbital shaking at 100 r.p.m.; the growth temperature was 26±1°C. The microalgal cells were grown in the batch culture for 14 days.

Stock solutions of the indole-3-acetic acid phytohormone (IAA – Sigma-Aldrich) were prepared in 98% EtOH. An ethanolic solution of IAA was added to the BBM medium in an amount to provide a final concentration of 10<sup>-4</sup> M and a final concentration of 10<sup>-5</sup> M in the second variant. Control samples did not contain IAA. All tested samples were inoculated with the same amount of sterile cell suspension of *P. kessleri* pre-culture. The initial optical densities (OD<sub>650</sub>) for the cultures were as follows: for the cultures growing under the photoperiod: 0.22 for the control: 0.20 for the concentration of 10<sup>-4</sup> M IAA, 0.20 for

the concentration of 10<sup>-5</sup> M IAA, and under continuous illumination: 0.17 for the control, 0.15 for the concentration of 10<sup>-4</sup> M IAA and 0.16 for the concentration of 10<sup>-5</sup> M IAA.

The experimental variants included cultivation of *P. kessleri* at constant 24-h illumination (experimental variant I) and at a 16/18 h light/dark cycle (variant II) under light intensity of 80 μmol photon m<sup>-2</sup> s<sup>-1</sup>. Each culture variant was performed in three biological replicates.

The growth of the *P. kessleri* culture was monitored daily for 14 days by spectrophotometric measurements of the optical density at 650 nm (OD<sub>650</sub>) using a Cary 300/Biomelt spectrophotometer. Based on the measurement of the optical density of the samples, growth parameters such as the specific growth rate and biomass doubling time were determined.

The specific growth rate ( $\mu$ ) (day<sup>-1</sup>) of *P. kessleri* microalgae was determined based on OD<sub>650</sub> using the following formula:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}, \quad (1)$$

where:  $N_1$  is the initial biomass concentration at time  $t_1$  and  $N_2$  is the biomass concentration at time  $t_2$  (Grudziński *et al.*, 2016).

The biomass doubling time  $T_d$  (h) of *P. kessleri* microalgae was determined based on the specific growth rate ( $\mu$ ) using the following formula (Piasecka *et al.*, 2014):

$$T_d = \left( \frac{\ln 2}{\mu} \right) 24. \quad (2)$$

The number of cells was determined based on direct counting in the Burkner chamber under the OLYMPUS/CKX41SF microscope.

For dry weight determination, the known volume of culture suspension was filtered through pre-weighed and pre-dried filters (47 mm glass fibre paper filter Whatman GF/C). Next, the filters were dried in a laboratory drier Venticell/VC111 at 100°C for 24 h to constant weight. The difference in the weight of the filters was converted to 1 l of a cell culture giving the content of dry cell weight in 1 l of *P. kessleri* culture.

Statistical analysis of the results was carried out on the basis of statistical calculations in the Statistica (version 12.5, StatSoft Inc., USA). Differences in the measured parameters were compared using one-way ANOVA followed by a Tukey HSD test.

#### RESULTS

The growth of *P. kessleri* culture differed and depended on the applied illumination and concentrations of the added indole-3-acetic acid (IAA) phytohormone from the auxin group.

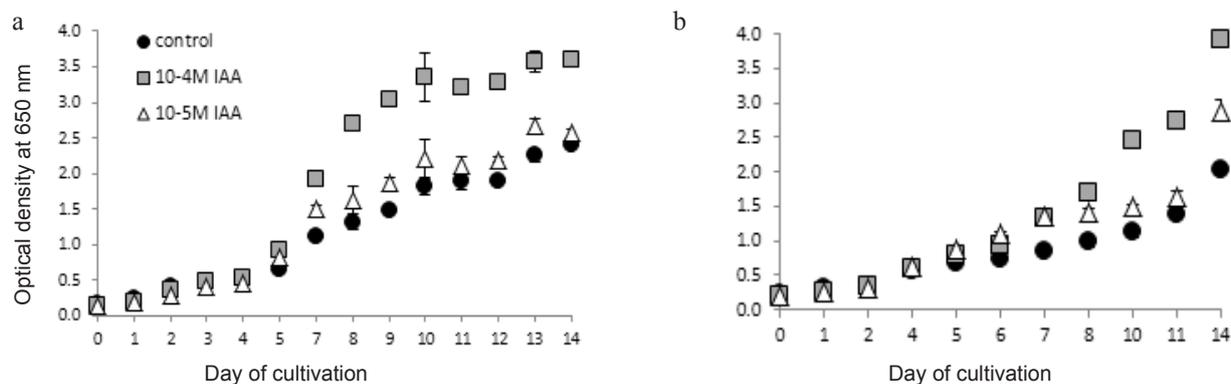


Fig. 1. Comparison of the growth of *P. kessleri* under the influence of IAA in: a – continuous illumination and b – photoperiod.

**Table 1.** The impact of the use of the photoperiod, continuous illumination, and the addition of IAA on growth parameters: specific growth rate and biomass doubling time of *P. kessleri*

Final concentration of IAA (mol dm <sup>-3</sup> )	Specific growth rate $\mu$ (day <sup>-1</sup> ) (0-8 days)	Biomass doubling time (h) (0-8 days)
Continuous illumination		
10 <sup>-4</sup>	0.37±0.02a	44.95±2.06a
10 <sup>-5</sup>	0.30±0.01b	55.57±2.72b
Control	0.25±0.01c	65.35±2.46c
Photoperiod		
10 <sup>-4</sup>	0.27±0.02a	61.52±3.72a
10 <sup>-5</sup>	0.24±0.01b	66.31±1.52b
Control	0.18±0.01c	93.88±2.73c

Different letters in columns indicate values significantly different \* $p < 0.01$ .

The comparison of the growth curve of *P. kessleri* is presented in Fig. 1. *P. kessleri* exhibited the lag growth phase between days 0-4 and next the linear phase was observed under continuous illumination and without IAA addition (control conditions) (Fig. 1a). The comparison of the light conditions applied revealed that the use of continuous illumination led to an increase in the values of OD<sub>650</sub> relative to the cultures growing under the photoperiod conditions. In the control conditions and at the photoperiod applied, *P. kessleri* cells were in the linear growth phase (Fig. 1b).

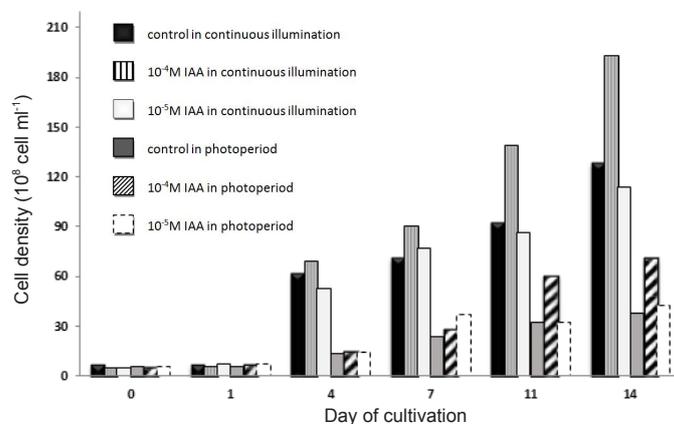
The addition of the IAA phytohormone had an impact on the duration of the phases of growth curves of *P. kessleri*. Under the 16/8 h light/dark photoperiod, the stationary phase was not observed regardless of the amount of the added phytohormone. Changes in the shape of the growth curve are visible after day 6 of the culture, where the addition of 10<sup>-4</sup> M IAA had a stimulatory effect on growth, and

the cells of *P. kessleri* entered the exponential phase earlier than the cultures containing a lower concentration of the phytohormone (10<sup>-5</sup> M IAA, Fig. 1b).

Under continuous illumination and at the higher concentration of the phytohormone (10<sup>-4</sup> M IAA), the cells of *P. kessleri* exhibited the exponential phase on days 5-10 and, next, the stationary phase was observed (Fig. 1a). The shape of the growth curve of the *P. kessleri* cultures supplemented with the lower concentration of IAA under continuous illumination was similar to the course of the growth curve in the cultures without the phytohormone addition. The OD<sub>650</sub> values were higher in cultures containing the higher concentrations of the phytohormone (10<sup>-4</sup> M IAA) than in those containing the lower concentration of the phytohormone (10<sup>-5</sup> M IAA).

The growth parameters of *P. kessleri* are summarized in Table 1. The indole-3-acetic acid (IAA) phytohormone has a stimulating effect on the growth of *Parachlorella kessleri* by increasing the specific growth rate and shortening the biomass doubling time relative to the control. The increase in the growth rate was proportional to the amount of the added phytohormone. A larger increase was observed upon addition of the higher concentrations of the phytohormone – 10<sup>-4</sup> M IAA than the lower concentrations – 10<sup>-5</sup> M IAA. At the 16/8 h light/dark photoperiod, the biomass doubling time was longer and the specific growth rate was lower than in cultures under continuous illumination. The highest growth rate ( $\mu = 0.37 \text{ day}^{-1}$ ) and the shortest doubling time (44.95 h) were observed under continuous illumination and the 10<sup>-4</sup> M IAA concentration (Table 1).

The addition of the phytohormone also resulted in an increase in the cell number in the *P. kessleri* culture, compared to the control (Fig. 2). At cultivation under continuous illumination, the number of *P. kessleri* cells in the control culture increased from  $7.10 \cdot 10^8$  to  $1.29 \cdot 10^{10}$  cells during 14 days of the culture. The highest growth of the cell number, *i.e.* from  $6.38 \cdot 10^8$  to  $1.93 \cdot 10^{10}$  cells, was observed in the culture containing 10<sup>-4</sup> M IAA. For the 10<sup>-5</sup> M concentration of IAA, a small increase in the cell number was observed, compared to the control, *i.e.* from  $6.83 \cdot 10^8$  to  $1.30 \cdot 10^{10}$  cells



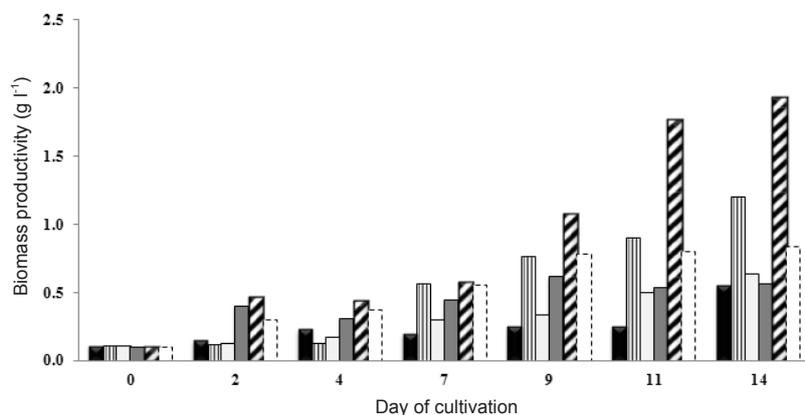
**Fig. 2.** Changes in the *P. kessleri* cell number induced by the addition of IAA under continuous illumination and the photoperiod.

over 1-14 days of cultivation. In the photoperiod cultivation, a decrease in the cell number was observed, compared to the culture under continuous illumination. The control cultivation of *P. kessleri* contained  $5.96 \cdot 10^8$  cells on day 1 of cultivation and  $3.76 \cdot 10^9$  cells on day 14. A slightly higher number of cells were noted in the culture with  $10^{-5}$  M of IAA, *i.e.* from  $7.21 \cdot 10^8$  to  $4.22 \cdot 10^9$  cells over 1-14 days of cultivation. The largest number of cells was found in the culture supplemented with  $10^{-4}$  M IAA, *i.e.*  $7.05 \cdot 10^8$  cells on day 1 and  $7.09 \cdot 10^9$  cells on day 14 of cultivation. However, compared to the culture in continuous illumination, the final number of cells grown in the photoperiod culture for 14 days was approximately 10-fold lower. The varied biomass productivity was dependent on the concentrations of IAA used (Fig. 3). The addition of the IAA phytohormone resulted in an increase in the production of *P. kessleri* biomass. This relationship was observed at both

the higher concentration of the phytohormone  $10^{-4}$  M IAA and the lower concentration  $10^{-5}$  M IAA. A higher final concentration of biomass was noted in the culture supplemented with the higher concentration of IAA ( $10^{-4}$  M IAA), compared to the culture containing the lower concentration of IAA –  $10^{-5}$  M.

#### DISCUSSION

Phytohormones are naturally occurring biostimulators identified in terrestrial plants, fungi, mosses. They are also found in some species of algae, but at lower concentrations (Kemal, 2002; Lau *et al.*, 2009; Lu and Xu, 2015; 2009; Yue *et al.*, 2014). There have been many available reports published over the years about the impact of phytohormones on the growth and development of terrestrial plants (Bari and Jones, 2009; Voeselek and Van der Veen,



**Fig. 3.** The influence of the photoperiod, continuous illumination, and IAA addition on *P. kessleri* biomass productivity (g l<sup>-1</sup>). Legend as in Fig. 2.

2014). However, the physiological, biochemical, and molecular roles of phytohormones in algae is not fully understood. In macroalgae, the role of phytohormones is probably similar to that in terrestrial plants. The effect of phytohormones in the case of microalgae is not so unequivocal (Mazur *et al.*, 2001; Tarakhovskaya *et al.*, 2013). In terrestrial plants, IAA is responsible for growth promotion, cell division and enlargement, and tissue differentiation (Zhao, 2010). The possible role of IAA in microalgae is discussed. There are reports in the literature that exogenous IAA-supplementation of the cultivation medium may play a role in the stimulation of microalgal growth (Gonzales and Bashan, 2000). It is known that each species of algae reacts differently to the same phytohormone (Salama *et al.*, 2014). However, not every concentration of phytohormones stimulates the growth of microalgae. Both too low and too high concentrations have a negative impact on the growth of algae. Therefore, it is highly important to find the optimal concentration of phytohormones for a particular species of algae. Physiological activities of IAA in relation to supporting cell division include response to light (Strik *et al.*, 2014). So far, there have been no reports in the literature about the effect of IAA addition on the growth of *P. kessleri*. There are also no studies on the cumulative effect of the addition of IAA in various illumination conditions.

The indole-3-acetic acid (IAA) phytohormone increased the growth of the *P. kessleri* microalgae. As shown in Table 1, the maximum specific growth rate was found for the concentration of  $10^{-4}$  M IAA in continuous illumination ( $0.37 \text{ day}^{-1}$ ), compared to the control ( $0.25 \text{ day}^{-1}$ ), which corresponded to the shortest biomass doubling time (44.95 h), compared to the control (65.35 h). In the same lighting conditions and the concentration of  $10^{-5}$  M IAA, the values of the specific growth rate were lower ( $0.30 \text{ day}^{-1}$ ) and the biomass doubling time was longer (55.57h) than for the concentration of  $10^{-4}$  M IAA. The literature shows that phytohormone supplementation, e.g. with indole-3-acetic acid and gibberellin (Lien *et al.*, 1971; Vance, 1987), auxins and cytokinins (Czerpak *et al.*, 1999), kinetin (6-furfurylamino-purine), significantly increases growth and cell division of *Chlorella sp.* (Li *et al.*, 2007).

In turn, under the photoperiod 16/8 h (light/dark) (Table 1), the specific growth rate was lower and the biomass doubling time was longer, compared to the corresponding concentrations in continuous illumination. This correlation is observed at both concentrations of IAA (respectively for  $10^{-4}$  M IAA:  $0.27 \text{ day}^{-1}$  and 61.52 h;  $10^{-5}$  M IAA:  $0.24 \text{ day}^{-1}$  and 66.31 h), compared to the control ( $0.18 \text{ day}^{-1}$  and 93.88 h).

Liu *et al.* (2016) observed an improved growth rate in the cultures of *Chlorella pyrenoidosa* supplemented with IAA (5.0 – 60 mg l<sup>-1</sup>) in BG 11 medium with a light cycle 12 h/12 h ( $100 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ) than in the treatment without IAA. The growth rate of *Scenedesmus quadricauda* in the presence of IAA added in the range of 5.0 – 60 mg

l<sup>-1</sup> was lower and its maximum value was  $0.64 \times 10^{-2} \text{ day}^{-1}$ ; in turn, it was  $0.65 \times 10^{-2} \text{ day}^{-1}$  for the IAA non-supplemented culture (Liu *et al.*, 2016). Piotrowska-Niczyporuk and Bajguz (2014) indicate that the growth of *Chlorella vulgaris* was stimulated at 0.1  $\mu\text{M}$  of IAA (under  $50 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$  light intensity and 16/8 h light/dark cycle); however, inhibitory effects were noted at a concentration of 100  $\mu\text{M}$  of IAA. Studies of exogenously added IAA (100  $\mu\text{M}$ ) under continuous illumination ( $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) showed no effect on the cell growth of *C. vulgaris* (Jusoh *et al.*, 2015). These results indicate that the biological response to supplementation of auxin maybe species dependent.

Salama *et al.* (2014) reported that IAA addition enhanced *Scenedesmus obliquus* growth under continuous illumination of light at  $45\text{-}50 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ . Among the tested concentrations of  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , and  $10^{-8}$  M, a maximum increase in *Scenedesmus obliquus* growth was observed at the concentration of  $10^{-5}$  M IAA. Microalgal growth increased with the increasing phytohormone concentrations. The maximum growth of *S. obliquus*, i.e.  $17.7 \times 10^6 \text{ cells ml}^{-1}$ , was observed on day 6 of cultivation. This constituted a 1.9-fold increase, compared to the control ( $9.5 \times 10^6 \text{ cells ml}^{-1}$ ). The present study has shown a 1.5-fold higher number of *P. kessleri* cells in the case of  $10^{-4}$  M IAA in continuous illumination, compared to the control ( $1.93 \times 10^{10} \text{ cells vs. } 1.29 \times 10^{10} \text{ cells}$ ) during 14 days of cultivation (Fig. 2). At the concentration of  $10^{-5}$  M of IAA, a slight increase in the number of cells was observed, compared to the control ( $1.30 \times 10^{10} \text{ cells}$ ). In the photoperiod cultivation, the greatest increase in the cell number was observed at the concentration of  $10^{-4}$  M IAA, compared to the control. For the concentration of  $10^{-4}$  M of IAA, a 1.9-fold higher number of *P. kessleri* cells was observed, compared to control ( $7.09 \times 10^9 \text{ cells vs. } 3.76 \times 10^9 \text{ cells}$ ) and only a 1.12-fold increase in the cell number was noted at  $10^{-5}$  M of IAA (Fig. 2). This study has shown 3.4-fold higher total biomass productivity of *P. kessleri* at the addition of  $10^{-4}$  M IAA in the 16/8 h light/dark photoperiod, compared to the control ( $1.93 \text{ vs. } 0.57 \text{ g l}^{-1}$ ) during 14 culture days, and 2.2-fold higher biomass productivity of *P. kessleri* at the supplementation with  $10^{-4}$  M IAA in continuous illumination, compared to the control ( $1.2 \text{ vs. } 0.55 \text{ g l}^{-1}$ ) (Fig. 3). The biomass productivity of *P. kessleri* obtained for the concentration of  $10^{-5}$  M IAA was 0.83 and  $0.63 \text{ g l}^{-1}$ , respectively, for the photoperiod and continuous illumination.

Park *et al.* (2014) examined the effect of various concentrations of IAA on biomass production in *Chlamydomonas reinhardtii*. They reported that the ca. 3 ppm concentration of IAA was optimal for *C. reinhardtii* growth stimulation, as it yielded a 61% increase in biomass production, compared to the control with maximum biomass production  $1.69 \text{ g l}^{-1}$ . The most favorable conditions for the biomass production in *P. kessleri* were provided by the 16/8 h light/dark photoperiod and the addition of  $10^{-4}$  M of IAA.

## CONCLUSIONS

1. The addition of indole-3-acetic acid increased biomass productivity of *Parachlorella kessleri*.

2. The higher concentration of indole-3-acetic acid ( $10^{-4}$  M) had a stimulating effect on the growth of *Parachlorella kessleri* microalgae in two variants of the lighting conditions applied.

3. *Parachlorella kessleri* show a maximum growth rate at continuous illumination and the concentration of  $10^{-4}$  M of indole-3-acetic acid.

4. The comparison of the culture conditions shows that continuous illumination and the concentration of  $10^{-4}$  M of indole-3-acetic acid promoted cell proliferation of *Parachlorella kessleri* more effectively than 16/8 h (light/dark photoperiod).

**Author contribution:** E. Magierek performed the experiments, analysed and interpreted the data, drafted the manuscript, and approved the version of manuscript to be submitted. I. Krzemińska devised the entire set of the experiments, analysed and interpreted the data, drafted the manuscript, made a revision based on other authors' contributions, approved, and submitted the final version of the article. J. Tys analysed the data and approved the version of the manuscript to be submitted.

**Conflict of interest:** The authors declare that there is no conflict of interest about the publication of this work.

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