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Culture density influence on the photosynthetic efficiency of microalgae growing under different spectral compositions of light

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ABSTRACT

A density in algal suspension causes a significant change in the intensity and spectral composition of light reaching individual cells. Measurements of chlorophyll fluorescence allow us to observe any general changes in the bioenergetic status of photosynthesis. The aim of the study was to determine the effect of cultivation density on the PSII photochemical efficiency of three species of algae (*Chlorella vulgaris*, *Botryococcus braunii* and *Chlorella emersonii*), each with a different rate of growth – high, medium and low – respectively. The cell density of algae in suspension differentiated through the cultivation time (2, 4, and 8 days) and the spectral composition of light. The results showed that the density of cultivation led to change in the photosynthetic apparatus of algae. The differences described between each day of cultivation (2, 4, and 8) in the kinetics of chlorophyll *a* fluorescence intensity in cells of the algal strains under study probably resulted from the different phases of growth of these cultures. In addition the results showed the beneficial effect of far red light on the photosynthetic apparatus and the growth of biomass in investigated algal strains.

1. Introduction

Light is one of the leading dynamically changing environmental factors. The changes are related to color, light intensity, direction of light, and variations in the length of light and dark periods of the diurnal cycle (photoperiodism). Plants only use a portion of the entire visible light spectrum for photosynthesis (within the range of 400–700 nm), which is called photosynthetic active radiation (PAR). However, an intensity of PAR too high or too low may become a stress factor, causing photoinhibition and the disruption of photosynthesis [1–3]. During the day the intensity of PAR is constantly changing, and plants try to maintain a balance between converting radiation energy and protecting the photosynthetic apparatus against photoinhibition and the repair of any potential resulting damage [4–6]. Terrestrial plants absorb the radiation most effectively in the blue (460 nm) and red ranges (660 nm), whereas, the green radiation is absorbed to a lesser extent. Both the spectral distribution and the light intensity in aquatic ecosystems are strongly modified by water and any substances it contains, forming a pervasive filter. A unique feature of an aquatic environment (compared to other environments) is its rapid disappearance from the range of deep far red. In spite of this, the photomorphogenetic mechanisms of aquatic ecosystems are similar to those of terrestrial ecosystems [7]. The vertical distribution of algae depends on the degree of its sensitivity to UV-B radiation, as the UV radiation affects many vital processes in algae [for review 8]. The upper stratum of the water column (photic zone) is inhabited predominantly by green algae, with brown algae predominately below in the dysphotic zone, where there is a limited amount of light, and red algae living in the bottom zone, where only green light is present.

Plants have two main light response systems (light perception): a photosynthetic system (including photosystem I - PSI, photosystem II - PSII) and a photomorphogenetic system. The function of the

Abbreviations: ABS/RC, light absorption flux (for PSII antenna chlorophylls) per reaction center (RC); Area, the area above the chlorophyll fluorescence curve between F0 and Fm (reflecting the size of the plastoquinone pool); Chl, chlorophyll; ChlF, chlorophyll *a* fluorescence; DI0/RC, dissipation energy flux per PSII reaction center (RC); ET0/RC, maximum electron transport flux (further than Q_A^-) per PSII reaction center (RC); F0, chlorophyll fluorescence intensity measured when all PSII reaction centers are assumed to be open, although the measured value may be affected by several other parameters (at t = 0); Fm, maximal chlorophyll fluorescence intensity measured when all photosystem II (PSII) reaction centers are closed; FRL, far red light; Fv/F0, a value that is proportional to the activity of the water-splitting complex on the donor side of the PSII; Fv/Fm, a value that is related to the maximum quantum yield of PSII; PI, performance indexes; R-FR, red-far red; Tfm, the time needed to reach Fm; TR0/RC, trapped (maximum) energy flux (leading to Q_A reduction) per reaction center (RC); VJ/RC, relative variable fluorescence at time J (relative variable fluorescence at phase J of the fluorescence induction curve).

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photosynthetic system is to capture light energy and process it into chemical energy by using photoreceptors, such as chlorophylls, carotenoids, and phycobilins. The amount of absorbed light quanta is the most important parameter here as it determines the intensity of photosynthesis, and thus the production of biomass. In algae [9,10], the degree of utilization of the individual colors of light is characterized by the unique phylum ratio of photoreceptors (pigments) present in the cells. Green algae, like taller plants, absorb mainly blue and red light (chlorophylls), while diatoms and brown algae absorb blue and green light (absorbed by carotenoids). In turn, red algae use yellow and green light most efficiently, being absorbed by phycobilins [11].

With regards to photomorphogenesis, energy is not a dominant factor, but the colors of light are (mainly blue, red, and far red). The information contained in UV-B radiation is also significant. The color of light is used by plants as a source of information about the ecophysiological conditions prevailing in the environment. The acclimation of plants to changing light conditions, which consists of receiving and reading the information contained in the light flux and processing it for a physiological response, allows the plant to function in an environment [12].

Measurements of ChlF (chlorophyll *a* fluorescence) allow us to observe any general changes in the bioenergetic status of photosynthesis. Furthermore, these kinds of measurements relate both directly and indirectly to all light-dependent stages of photosynthesis, including water decomposition, electron transport, pH gradient formation across the thylakoid membrane, and ATP synthesis. This is a good method for assessing the impact of stress factors on the process of photosynthesis. It is a simple, non-invasive, inexpensive, and fast tool for analyzing the photochemical reactions of photosynthesis. Improved techniques of ChlF measurements allow us to study the process of photosynthesis at different functional levels (e.g., light collecting complexes, basic light reactions, electron transport chains, light-independent reactions in thylakoids, and even slow regulatory processes) [13–16]. The parameter values obtained from the analysis of the ChlF kinetics can be used directly to test the reaction of photosynthesis under adverse environmental conditions and, indirectly, to assess the condition of plants [17]. Chlorophyll *a* fluorescence parameters are a useful tool for monitoring the PSII reaction to changes in light intensity [18,19] and spectral composition [20]. The appropriate selection of ChlF techniques allows us to determine the PSII response to different environmental conditions, including nutrient deficiency, salinity, heavy metals, the quality and intensity of PAR, high and low temperatures, ozone, and herbicides [21–23]. Tests may be conducted on a single plant (including transgenic and cultures in vitro) or on an entire ecosystem, in order to estimate the potential performance and tolerance to stress factors of individual or groups of organisms. The JIP test is a reliable mathematical model suggested by Strasser et al. [24] that provides detailed information on the structure and function of the photosynthetic apparatus and enables a quick estimate of plant vitality. It based on analyzing the induction curve of chlorophyll *a* fluorescence between O (10 us) and P (300 ms) bands.

The majority of studies performed so far on algae have focused either on the response of the photosynthetic apparatus to the stressor (temperature, herbicides, heavy metals, etc.) or on the spectral composition of light. These studies, however, were most frequently conducted at one point in time (usually at the end of cultivation), while there were a rather small number of dynamic studies describing the changes in the functioning of the photosynthetic apparatus at a subsequent point in cultivation. Such studies would answer the question of whether and how changes in the functioning of the photosynthetic apparatus depend on the number of cells produced during cultivation, and thus on the amount of biomass. A density in suspension causes a significant change in the intensity and spectral composition of light reaching individual algal cells. This is because an increasing number of cells during cultivation changes with their length of time in light and in darkness (periods of changing from the shade to light) and obviously, with the spectral composition of the light reaching them. Therefore, it can be supposed that the photosynthetic apparatus of algae must be continuously adapting to the changing light conditions in cultivation.

The aim of the study was to determine the effect of cultivation density on the photochemical efficiency of PSII. The cell density of algae in suspension differentiated through the cultivation time (2, 4, and 8 days) and the spectral composition of light. The experiment was performed on three species of algae (*Chlorella vulgaris*, *Chlorella emersonii* and *Botryococcus braunii*), each with a different rate of growth. This allowed for additionally differentiating the densities of suspensions in various stages of growth (time/spectral composition).

2. Materials and Methods

2.1. Microalgae

Three strains have been selected for the study of the phylum *Chlorophyta* (green algae), class Trebouxiophyceae. These were *Chlorella vulgaris*, *Chlorella emersonii*, and *Botryococcus braunii*. The strain of *C. vulgaris* was obtained from the Bohdan Dobrzański Institute of Agrophysics of the Polish Academy of Sciences in Lublin, Poland. The strains of *C. emersonii* and *B. braunii* were purchased from the SAG collection in Gottingen, Federal Republic of Germany (SAG 2334, SAG 807 – 1, respectively).

2.2. Growth Conditions

C. vulgaris was cultivated in a medium described by Kessler and Czygan [25], *C. emersonii* on the AF6 medium [26], while *B. braunii* on the BG11 medium [27].

The cultivation was carried out in SARTORIUS BIOSTAT® PBR 2S algae bioreactors with a capacity of 3 dm^3. For the algae culture 2.9 dm^3 of medium was used, appropriate for the given strain, to which 0.1 dm^3 of the algal suspension was added. The cultures were grown for 8 days at 25 °C for *C. vulgaris* and 30 °C for *C. emersonii* and *B. braunii*, at a photoperiod of 16/8 h (light/dark). The measurements were performed on days 2, 4, and 8 of cultivation. The O_2 concentration in the bioreactor was maintained at full saturation (for the set temperature and at the normal atmospheric pressure) by removing the excess O_2 (arising from algae photosynthesis) by blowing in pure N_2. While at night, when the oxygen concentration was decreasing (due to intense dark respiration), the cultures were oxygenated with pure O_2 equal to the value of their initial concentrations. The CO_2 concentration was controlled by maintaining the pH of the medium by pulse CO_2 addition at the time of pH increase. All parameters were automatically controlled and operated by the photobioreactor.

2.3. Light Sources

The light sources were analogous to those previously reported [28]. Briefly, white light (WL) was provided by fluorescent lamps of 18W/840, while blue-red light (B-RL) and blue-red-far red (B-R-FRL) were obtained from the LED matrix, which consisted of three colors: blue, red and far red.

2.4. Determination of Algal Biomass

The growth dynamics of the algal biomass was tested by taking 5 ml of cell suspension from the culture on days 2, 4, and 8. The samples were centrifuged in a Hettich-Universal 32R centrifuge for 15 min at 2000 × *g*. The supernatant was discarded and the pellets were washed with 3 ml of distilled water and centrifuged again under the conditions described above. After centrifugation, the pellets were lyophilized for 48 h. The weight samples were determined within an accuracy of 10^{-6} g.

2.5. Photosynthetic Efficiency

The performance of PSII (photosystem II photochemical efficiency) was determined by measuring the chlorophyll *a* fluorescence (ChlF) parameters using a Handy Plant Efficiency Analyzer apparatus (Handy PEA) with an attachment used for liquid samples (Hansatech Instruments Ltd., United Kingdom) at room temperature. The intensity of the excitation light was set to 1500 μmol photons m^{-2} s^{-1}. Two milliliters of cell suspension collected directly from the cultures was added to the measuring cuvette. The measurements were performed after a 6-minute adaptation of the sample to darkness. Adaptation time was optimized experimentally before the start of the experiments. Kinetic fluorescence parameters were recorded within the range of 10 μs to 1 s. The following parameters were analyzed, based on the results obtained: TR0/RC, ET0/RC, DI0/RC, VJ/RC, F0, Fm, Fv/Fm, PI, Area, Fv/F0, Tfm, ABS/RC [29]. The analysis of the relationship between the structure and function of the photosynthetic apparatus and the assessment of the vitality of the algae was based on the JIP-test [24,30]. Microsoft Excel 10.0 was used to perform the analysis and draw the charts.

2.6. Fluorescence Emission Spectra

The room temperature fluorescence emission spectra were recorded with a fluorescence spectrometer Perkin-Elmer LS 50N (United Kingdom). The fluorescence spectrum was measured in 3 ml of cell suspension taken directly from the bioreactor to the measuring cuvette. Before measurement, the cuvette was placed in the dark for 5 min in order to achieve complete PS relaxation. A magnetic stirrer was used in order to prevent sedimentation of the cells at the time of measurement. The fluorescence intensity in the R-FR range (600–800 nm) was measured with 430 nm excitation using a 15 nm and 20 nm light path for the excitation and emission monochromators, respectively. The emissions, at 685 nm, 695 nm, 725 nm, and 735 nm, were considered to be fluorescence from the PSII antenna (PSIIA), the PSII core (PSIIC), the PSI antenna (PS IA), and the PSI core (PSIC), respectively [31,32]. The FL WinLAb Version No. 3.00 program was used to operate the spectrofluorometer and to analyze the results.

2.7. Statistical Analysis

The results presented in the study represent the mean of 10 independent biological replicates ± SD. The comparison of the significance of differences in mean values between objects was performed using the Fisher's NIR test procedure at the $p \leq 0.05$ level of significance.

The statistical analysis was performed using STATISTICA (data analysis software system), version 12 StatSoft, Inc. (USA) (2014) www.statsoft.com.

3. Results

3.1. Determination of Algal Biomass

To better illustrate the differences in biomass growth rate in the test strains, biomass values are shown in the same scale.

Irrespective of algae the strain and spectral composition of light, the increase in biomass up to day 4 of cultivation was very low (Fig. 1B and C) or not present at all, as in the case of *C. vulgaris* (Fig. 1A). However, the biomass of *C. vulgaris* and *B. braunii* on day 8 was higher (7- or 8-fold) in comparison with that observed on day 4 (Fig. 1A and C). The *C. emersonii* biomass on day 8 was only 1.5-fold higher, or did not differ under B-R-FRL, when compared to day 4 of cultivation (Fig. 1B).

Spectral light composition exerted a significant influence on the growth of the biomass. This effect, however, varied in the case different strains. In *C. vulgaris* the spectral composition of light had no effect on biomass growth up to day 4 of cultivation. This effect appeared only on day 8 of cultivation, when the largest increase in biomass was observed in cultures grown under WL, and the lowest under B-RL (Fig. 1A). In the case of *C. emersonii* the effect of spectral light composition on biomass growth was visible in each day of measurement. In the early stages of cultivation (days 2 and 4) a significantly higher biomass growth was recorded in cultures grown under B-R-FRL. After 8 days the highest increase in the biomass was recorded under WL (approx. 1.5-fold) while, under B-RL and B-R-FRL, the amount of biomass remains the same (Fig. 1B). On the second day of cultivation *B. braunii* biomass differed due to the spectral composition of light between the cells growing under B-RL and B-R-FRL and those under WL, where the lowest increment was observed. On day 4 the largest increase in biomass was under B-R-FRL, which was significantly different from the culture grown under WL. Similarly, as with *C. vulgaris*, a significant increase in biomass was observed on day 8 of cultivation when compared with that of earlier periods. On this day, the greatest amount of biomass was found in cultures grown under B-R-FRL and B-RL, and slightly lower under WL (Fig. 1C).

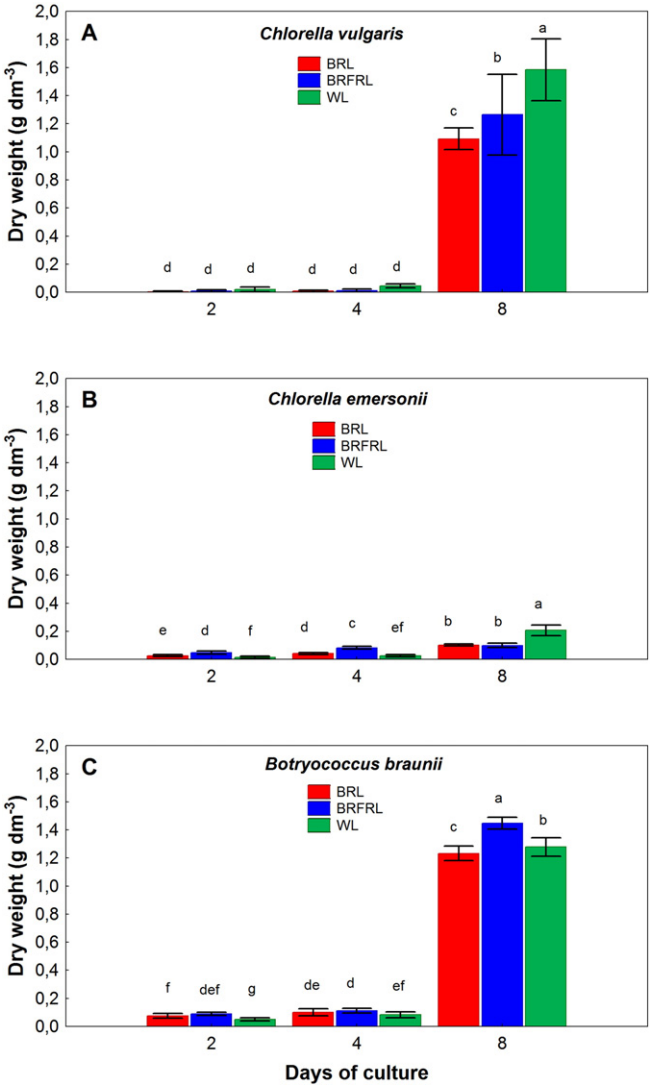

Fig. 1. Effect of spectral light compositions on algal biomass: Changes in the levels of biomass in *Chlorella vulgaris* (A), *Chlorella emersonii* (B) and *Botryococcus braunii* (C) on days 2, 4 and 8 of culture in different compositions of spectral light (white light – WL, blue-red light – B-RL and blue-red-far red light – B-R-FRL). The mean values of 6 replicates ± SE. The values marked with different letters are significantly different according to Fisher's NIR test at $p \leq 0.05$.

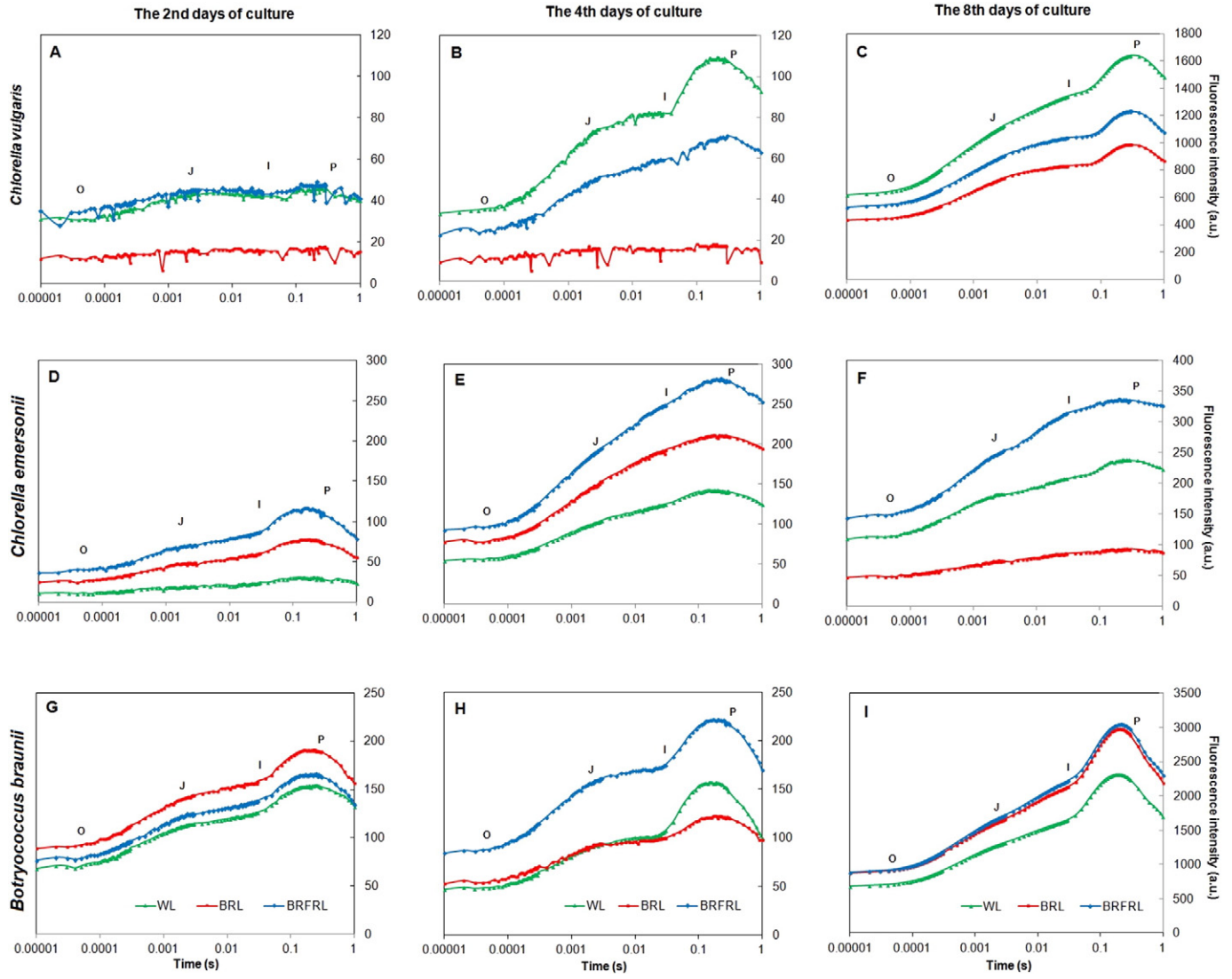

Fig. 2. Effect of spectral light compositions on chlorophyll *a* fluorescence induction curves (marked with particular OJIP phases at 3000 μmol photon m^{-2} s^{-1}) for *Chlorella vulgaris* (A–C), *Chlorella emersonii* (D–F) and *Botryococcus braunii* (G–I) on days 2, 4 and 8 of culture, respectively, in different compositions of spectral light (white light – WL, blue-red light – B-RL and blue-red-far red light – B-R-FRL). The mean values of 6 replicates.

3.2. Photosynthetic Activity

The analysis of ChlF kinetics of algal suspensions growing under different spectral light compositions was performed separately for three time points, i.e., days 2, 4, and 8 of cultivation (Fig. 2A–I and Fig. 3A–I). The results of the analysis of selected fluorescence parameters are shown on spider plots as control percentages, assuming 100% for the values obtained under WL as the most similar to the composition of solar radiation. To better illustrate the differences in the activity of PSII between objects, scale values on JIP-test and spider plots were selected individually for each strain and day of cultivation.

All abnormalities in either the structure or functioning of the photosynthetic apparatus were visible in the form of changes to the induction curve (Fig. 2). The analysis of fluorescence in the O, J, I, and P bands (JIP-test) made it possible to precisely examine the physiological condition of the photosynthetic apparatus and to assess plant vitality on this basis [30]. In the growth period under study, regardless of the spectral composition of light, there was a significantly greater increase in fluorescence intensity in *C. vulgaris* and *B. braunii* (Fig. 2A–C and G–I) than in *C. emersonii* (Fig. 2D–F). The greatest differences were observed between days 4 and 8 of cultivation, when fluorescence intensity in *C. vulgaris* and *B. braunii* increased by ten times (Fig. 2B–C and H–I).

On the 2nd day of cultivation, the three species showed weak fluorescence intensity, though most prominently in *C. vulgaris*. Furthermore, the shape and fluorescence intensity of the OJIP fluorescence transients recorded at different time periods of cultivation differed among algal cell species. In addition, light source seemed to have a significant effect on the OJIP fluorescence transient. This effect was also manifested as a change in the biomass during cell growth. For instance, after 8 days of cultivation, O-J, J-I and I-P phases were distinguishable in *C. vulgaris* cultivated under WL and in *C. emersonii* cultivated under B-R-FRL. The OJIP curve in the case of the *C. vulgaris* culture growing under B-RL had the lowest values throughout the whole period of growth. At day 4 of cultivation, clear changes occurred in the course of OJIP curves for individual cultures, depending on the color of light. The intensity of maximum fluorescence (with a strongly marked phase P) was the highest under WL, while on day 2, there were no changes in fluorescence intensity and OJIP induction curves for algae growing under B-RL (Fig. 2B). On day 8 of cultivation, the shape of the curves under discussion was similar in all spectral light compositions. However, it should

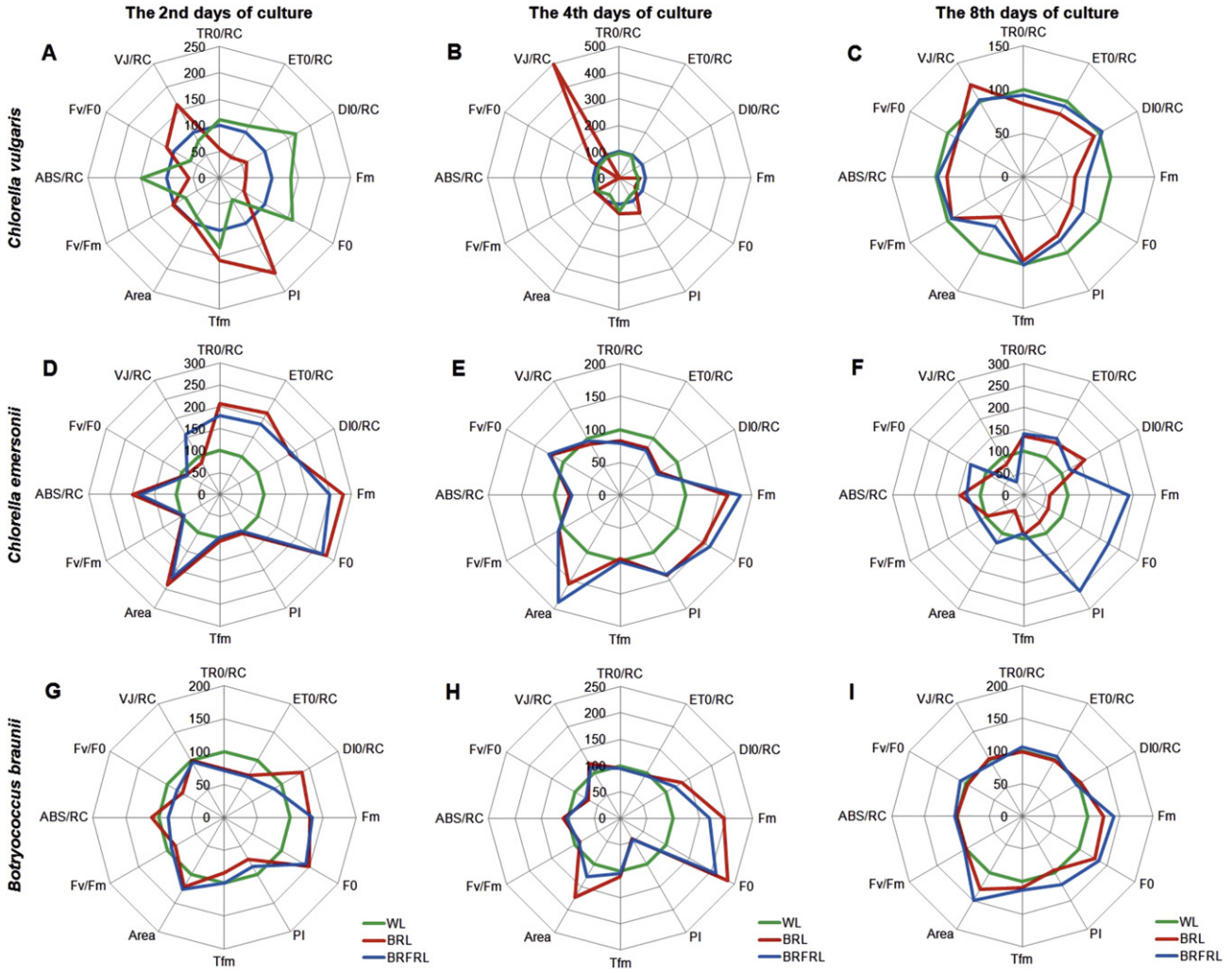

Fig. 3. Changes of shape of spider plot OJIP-test parameter images induced by different spectral composition and day of culture of *Chlorella vulgaris* (A–C), *Chlorella emersonii* (D–F) and *Botryococcus braunii* (G–I).

be noted that there was a significant change during the course of the I-J phase for algae cells under WL and changes throughout the entire course of the OJIP curve for cells under B-RL as compared to day 4 (Fig. 2C).

In *C. emersonii*, fluorescence intensity was relatively low during the growth period under analysis. The greatest ChlF intensity was observed on both days 2 and 4 in cells growing under B-R-FRL, and the least under WL (Fig. 2D and E). The intensity of ChlF increased two-fold on day 4 (Fig. 2E), while on the 8th day of cultivation a decrease in the value of the OJIP curve (by more than half) was observed in the cells under B-RL and an increase was recorded under all other light spectra. In addition, the difference in fluorescence intensity decreased between phases I and P on the OJIP curve for cells from B-R-FRL, reaching a plateau, as compared to curves observed earlier (Fig. 2F).

In *B. braunii*, irrespective of the spectral composition of light, the shape of the curves and their values were comparable on day 2 (Fig. 2G). On day 4 of cultivation, an increase was observed in the value of the OJIP curve in cells grown under B-R-FRL, with clearly marked O-J and I-P phases. In contrast, OJIP curves for cells under B-RL had significantly lower values, and consequentially, they were very similar to those under WL, except for the I-P phase (Fig. 2H). On day 8, the OJIP curves of the cells cultivated under B-RL and B-R-FRL were the same both in terms of shape and absolute value. The OJIP curve for cells grown under WL was similar in shape to those from B-RL and B-R-FRL, however, the intensity of ChlF for all phases was significantly lower (Fig. 2I).

In order to further demonstrate the effect of light source on PSII during algal cell cultivation, certain photosynthetic parameters were used to analyze PSII behavior and activity (Fig. 3 A-I). As shown in Fig. 3A, the fluorescence parameters Fv/Fm and Area not significantly changed in *C. vulgaris* two days after inoculation. In contrast, the other parameters under study were significantly changed (i.e., F0, Fm, PI, Tfm, ABS/RC, VJ/RC, and DI0/RC). However, this change was dependent upon the spectral composition of light e.g., PI and VJ/RC increased under B-RL but decreased under B-R-FRL. Parameters such as F0, Fm, ABS/RC and DI0/RC had higher values under B-R-FRL and lower values under B-RL (Fig. 3A). On the 4th day, a significant change was observed in VJ/RC and PI; these two parameters increased in the amount of algal cells cultivated under B-RL. The parameters, the values of which were higher under B-R-FRL in the second day of cultivated, were comparable or lower than under WL (Fig. 3B). Surprisingly, on the 8th day the photosynthetic parameters calculated for *C. vulgaris* cells cultivated either under B-RL or B-R-FRL showed a similar pattern of change, as under both light conditions a significant decrease was observed in Fm, F0, PI and Area (Fig. 3C).

Except for PI, Tfm and Fv/Fm, most of the photosynthetic parameters in *C. emersonii* exhibited a significant increase on the 2nd day. We noted here that the trend of change in these parameters was the same on the

2nd day under B-RL and B-R-FRL, with the exception of VJ/RC, which decreased under B-RL (Fig. 3D). On day 4 similar changes were observed in algae growing under B-RL and B-R-FRL, as compared with the control (WL). The values of the parameters describing the energy flow (TR0/RC, ET0/RC and DI0/RC), and ABS/RC were lower than those under WL. This indicated the difference in PSII behavior at the second day of cultivation (Fig. 3E). However, on the 8th day, PSII activity, measured as a change in fluorescence parameters, exhibited some specific effects with respect to light spectra. For example, Fm, F0 and PI increased in algal cells grown under B-R-FRL, but these parameters were reduced when the algal cells were grown under B-RL. In addition, both an increase in energy flow and ABC/RC parameters were again observed (Fig. 3F).

Interestingly, the behavior of PSII activity was the same in *B. braunii* cultivated under B-RL and B-R-FRL during the cultivation period under analysis. In the second day of cultivation, the PI, Fv/F0, TR0/RC and ET0/RC parameters were lower in the cells grown under B-RL and B-R-FRL than under WL, whereas parameters such as Fm, F0 and Area were higher under B-RL and B-R-FRL than under WL (Fig. 3G). These changes were significantly more visible on the 4th day of cultivation (Fig. 3H). On day 8 almost all parameters of PSII activity were similar under every spectral composition of light. The values of F0, Fm and Area parameters were still higher under B-RL and B-R-FRL when compared with WL. In addition, the culture growing under B-R-FRL has the highest PI value (Fig. 3I).

3.3. Fluorescence Emission Spectra

It was shown that the fluorescence intensity in the red – far red range, calculated at the peak maximum of approx. 680 nm, correlated with the amount of algal biomass ($R = 0.710$ $p < 0.000000$; $F(1.79) = 80.5$). Therefore, the determination coefficient ($R^2 = 0.498$) indicated that approximately 50% of fluorescence intensity variation could be explained by changes in the amount of biomass. Hence, the emitted fluorescence intensity was recalculated for each strain and day of cultivation per mass unit. Therefore, the results plotted reflected changes in fluorescence resulting from the length of cultivation and the color of light, excluding the influence of the amount of biomass. The scale values plotted were chosen to emphasize the aforementioned differences between the objects.

Chlorophyll fluorescence emission spectra of algal cultures produced a peak in the red portion of the spectrum with a maximum peak of between 680 and 690 nm (determined as F690) and a shoulder measuring near 735 nm (F735).

The tested strains of algae differed in their intensity of fluorescence due to the age of the culture (day of cultivation) and the color of light. With regards to *C. vulgaris* and *B. braunii*, on day 2 these cultures showed the highest fluorescence values (Fig. 4A and G), while fluorescence intensity subsequently decreased When compared to the 2nd day of cultivation, it differed on day 8 by 100 times from the amount of that in *C. vulgaris* and by 10 times from the amount of that in *B. braunii*. It should be noted that the cells of *C. vulgaris* and *B. braunii* had, on average, a similar fluorescence intensity on day 8 of cultivation (Fig. 4C and I). However, in *C. emersonii* there were no such differences in the fluorescence intensity of the cells during the period of time under analysis (Fig. 4D–F).

With respect to *C. vulgaris*, the lowest fluorescence intensity was observed throughout the whole experimental period of growth under WL (Fig. 4A–C). Fluorescence intensity values under WL were from 5 to 2 times lower, respectively, when compared with other configurations

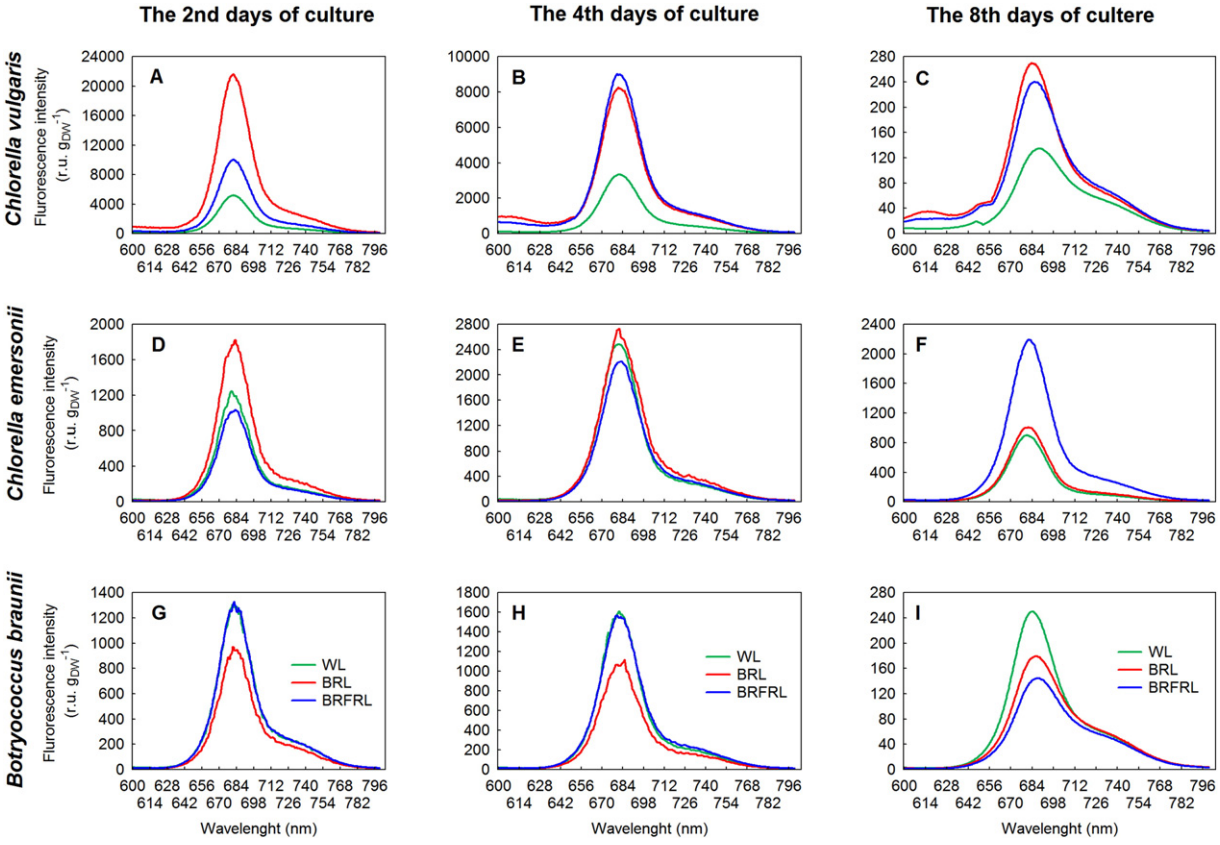

Fig. 4. Specific emission fluorescence spectra (r.u. g_{DW}^{-1}) of *Chlorella vulgaris* (A–C), *Chlorella emersonii* (D–F) and *Botryococcus braunii* (G–I), on days 2, 4 and 8 of culture, respectively, under white light (WL), blue-red light (B-RL) and blue-red-far red light (B-R-FRL). The mean values of 6 replicates.

of spectral light. On day 2 of growth under B-RL, fluorescence intensity was 2.5 times greater than under B-R-FRL (Fig. 4A). Subsequently, the fluorescence intensity under both spectral compositions became very similar (Fig. 4B and C). The greatest fluorescence in *C. emersonii* was observed on the 2nd day under B-RL, while under B-R-FRL it was on the 8th day. On day 2 the fluorescence intensity of cells grown under WL was similar to that under B-R-FRL, and on day 8 it was similar to B-RL (Fig. 4D and F). On day 4 the fluorescence of algae cells was very similar for all spectral light compositions (Fig. 4E). In *B. braunii* fluorescence intensity on both days 2 and 4 was very similar, depending on the spectral composition of light. This observation applies both to the absolute values of fluorescence intensity and to the interrelationships between the objects (Fig. 4G and H). On day 8 the highest fluorescence value was still observed in the cultures growing under WL, and the lowest under B-R-FRL (Fig. 4I).

The present study investigated the effect of time of cultivation and the spectral composition of light on algal photosystems (PS). Therefore, fluorescence intensity emitted by the antennas (A) and PSI and PSII core (C) in the algae cells was measured in the range between 600 nm and 800 nm after excitation with light of a 440 nm wavelength.

For *C. vulgaris*, statistically significant differences were found for all PSI and PSII fluorescence parameters, depending on the day of cultivation and the spectral composition of light. However, the spectral composition significantly affected the value of fluorescence parameters only on the 2nd and 4th days. Particularly noteworthy is the fact that in the culture growing under WL all test parameters (except for PSI/PSII) were lower than those observed in other spectral compositions (B-RL and B-R-FRL) on both the 2nd and 4th days (Table 1). On day 8 of cultivation the parameters discussed, except for PSII A/C and PSI/PSII ratios, did not exhibit a dependency on the spectral composition of light. It can be seen that the PSII A/C value was the highest under B-RL and the lowest under WL (Table 1). In *C. emersonii* PS fluorescence parameters for all objects growing under B-R-FRL were significantly different when compared with other objects (WL and B-RL). In comparison with other objects, A and C fluorescence emission intensities for PSII and PSI under B-R-FRL were low on days 2 and 4 and high on day 8. Furthermore, the PSII A/C ratio under B-R-FRL remained constant during subsequent days of growth (Table 1). In *B. braunii* the values for all parameters measured were mainly dependent on the growth phase (day of cultivation), while there was no variation observed in relation to the spectral composition of light (Table 1).

4. Discussion

The results showed that the photosynthetic apparatus of algae was constantly acclimating to changing (due to the increasing density of cultivation) light conditions. These changes, however, required time, since the density alone did not directly affect the efficiency of PSII. The experiments conducted so far on *C. vulgaris* suspension with different densities (expressed as OD_{750}) obtained by means of successive dilutions, have shown that only ChlF kinetic parameters, such as F0 and Fm, depended on a rapidly changing density. The values of other ChlF kinetic parameters and electron flux parameters did not depend on the amount of biomass obtained during 8-day periods of cultivation (OD_{750} 0.12 to 1.00, see: Supplementary materials).

The evaluation of the overall efficiency of photosynthesis on different days of cultivation for the three strains of algae under different spectra of light was based on measurements of in vivo transient ChlF [10].

The phases of growth and light quality affected the transport of electrons in the PSII as well as the intensity of ChlF in the JIP-test [33]. Increasing the intensity of fluorescence measurements in the following days of *C. vulgaris* and *B. braunii* shows an increase in biomass and a

Table 1
Photosystem II (PSII) and photosystem I (PSI) fluorescence of *Chlorella vulgaris*, *Chlorella emersonii*, and *Botryococcus braunii* cells growing at a different light spectral composition after 2, 4, and 8 days of growth. The fluorescence peaks of the antenna (A) and core (C) regions of the photosystems were used to calculate the ratio.

Days of cultivation	Spectral composition	PSII A	PSII C	PSII A/C	PSI A	PSI C	PSI A/C	PSI/PSII
Chlorella vulgaris								
2	WL	3985^{d}	2476^{d}	1.61^{ab}	581^{c}	482^{d}	1.20^{ab}	0.75^{c}
	B-RL	28927^{a}	17454^{a}	1.66^{a}	4143^{a}	3386^{a}	1.23^{a}	0.76^{c}
	B-R-FRL	10932^{b}	6868^{b}	1.59^{b}	1297^{b}	1319^{b}	1.18^{b}	0.73^{c}
4	WL	2859^{de}	1597^{de}	1.62^{ab}	431^{cd}	332^{de}	1.22^{a}	0.75^{c}
	B-RL	7394^{c}	4813^{c}	1.57^{b}	1165^{b}	929^{c}	1.23^{a}	0.78^{c}
	B-R-FRL	8800^{bc}	5560^{bc}	1.58^{b}	1331^{b}	1102^{bc}	1.21^{ab}	0.76^{c}
8	WL	164^{e}	150^{e}	1.10^{e}	64^{d}	55^{e}	1.17^{b}	1.07^{a}
	B-RL	305^{e}	246^{e}	1.32^{c}	80^{d}	68^{e}	1.19^{ab}	0.95^{b}
	B-R-FRL	241^{e}	205^{e}	1.17^{d}	78^{d}	66^{e}	1.18^{b}	1.00^{b}
Chlorella emersonii								
2	WL	1293^{bc}	807^{bc}	1.61^{bc}	195^{bc}	49^{bcd}	1.26^{a}	0.80^{a}
	B-RL	1878^{b}	1190^{b}	1.60^{bc}	273^{b}	239^{b}	1.15^{b}	0.72^{bcde}
	B-R-FRL	569^{c}	366^{c}	1.57^{cd}	67^{c}	59^{d}	1.13^{b}	0.76^{abc}
4	WL	3427^{a}	2050^{a}	1.68^{ab}	467^{a}	391^{a}	1.19^{b}	0.71^{cde}
	B-RL	3274^{a}	2211^{a}	1.49^{d}	520^{a}	452^{a}	1.16^{b}	0.78^{ab}
	B-R-FRL	1615^{bc}	1042^{bc}	1.56^{cd}	260^{b}	217^{bc}	1.13^{b}	0.75^{abcd}
8	WL	783^{bc}	446^{bc}	1.76^{a}	93^{bc}	79^{cd}	1.18^{b}	0.67^{e}
	B-RL	1590^{bc}	951^{bc}	1.68^{ab}	217^{bc}	182^{bcd}	1.18^{b}	0.70^{de}
	B-R-FRL	3440^{a}	2442^{a}	1.57^{cd}	546^{a}	472^{a}	1.20^{b}	0.76^{abc}
Botryococcus braunii								
2	WL	1230^{bc}	847^{abc}	1.46^{b}	236^{a}	203^{a}	1.15^{ab}	0.79^{cd}
	B-RL	1019^{c}	695^{c}	1.47^{b}	211^{a}	183^{a}	1.15^{ab}	0.78^{cd}
	B-R-FRL	1159^{c}	792^{bc}	1.46^{b}	230^{a}	191^{a}	1.20^{a}	0.82^{c}
4	WL	1793^{a}	1067^{a}	1.67^{a}	255^{a}	226^{a}	1.13^{b}	0.68^{d}
	B-RL	1350^{abc}	815^{abc}	1.69^{a}	197^{a}	173^{a}	1.14^{ab}	0.69^{de}
	B-R-FRL	1615^{ab}	1008^{ab}	1.60^{a}	256^{a}	221^{a}	1.16^{ab}	0.73^{cde}
8	WL	322^{d}	247^{d}	1.28^{c}	82^{b}	70^{b}	1.18^{ab}	0.92^{b}
	B-RL	261^{d}	220^{d}	1.15^{cd}	86^{b}	73^{b}	1.17^{ab}	1.02^{ab}
	B-R-FRL	198^{d}	175^{d}	1.10^{d}	74^{b}	63^{b}	1.16^{ab}	1.05^{a}

Note: For each strain of algae, different letters in the same column are significantly different at $p \leq 0.05$ according to Fischer's NIR test. The number of replicates was 6 (for each spectral composition of light). Peak fluorescence was measured at 685 nm (PSIIA), 695 nm (PSIIC), 725 nm (PSIA), and 735 nm (PSIC) of excitation with a 440 nm monochromatic light.

phase change in cell growth. The low intensity fluorescence is characterized by the cells in the lag phase (Fig. 2A, B, G and H), whereas the high fluorescence intensity is connected with a logarithmic (exponential) phase of growth (Fig. 2C and I). However, in the case of *C. emersonii*, a slight increase in fluorescence intensity between the 2nd and 8th days and thus the cells might be in the same initial phase (lag phase) of growth (Fig. 2D–F).

The irregular course of OJIP curves on days 2 and 4 of *C. vulgaris* cultivation was probably due to the low measure of the signal-to-noise ratio at a low ChlF (Fig. 2A and B). The change in the shape of OJIP curves and the signal stability in the 4th day of *C. vulgaris* cell culture grown under WL and B-R-FRL showed that the aforementioned light conditions were more favorable for cell growth and functioning of the photosynthetic apparatus than was B-RL.

As shown by the OJIP curves, depending on the strain, B-RL adversely affected the photosynthetic apparatus in the entire growth period for *C. vulgaris*, on day 8 for *C. emersonii*, and day 4 for *B. braunii*. The shape of curves in the JIP-test depends both on the course of photosynthetic electron transport from the PSII reaction center as well as on a reduction of the QA electron acceptors PQ pool in PSII. The decrease in fluorescence intensity in phases I and J resulted from the inhibition of electron transport to the PSII donor side, which led to the accumulation of $P680^{+}$, which is a potent fluorescence quencher. Additionally, these changes described a lower electron contribution from the oxidized portion of the photosynthetic apparatus and a strong inhibition of electron transport from the water splitting complex to the PQ pool. The decrease in the intensity of the phase I-P indicates, in turn, a reduction in the activity of PSI [33]. Moreover, large differences were observed in the value of the P point on the OJIP curve (Fm value) in all tested cultures, dependent on both the strain and day of cultivation as well as on the color of light (Fig. 2A–I). Low Fm values can result from a strong inhibition of the acceptor side of PSI [34]. On the other hand, Schansker et al. [35] reported that the smaller size of PSII antennae could slow down the reduction of the PQ pool, which consequentially might also result in lower Fm values. In the case of *C. vulgaris*, low Fm values were observed in cultures grown under B-RL throughout the growth period (Fig. 2A–C). This may indicate that B-RL in *C. vulgaris* may adversely affect the formation of PSII antennae. At the same time, much higher Fm values under WL indicated that this spectral composition seemed to be optimal for the functioning of the photosynthetic apparatus and the increase in biomass as a result. A decrease in Fm values and a suppression of the I-P phase in the fluorescence rise kinetics observed on day 8 of the *C. emersonii* cultivation as compared to day 4 (Fig. 2E and F) indicated a decrease in PSI activity under the growth conditions mentioned earlier [34]. The analysis of the kinetics parameters of ChlF in *B. braunii* showed that under WL both F0 and Fm had lower values when compared with B-R-FRL (Fig. 2G–I). It seems that with respect to algae growing under WL, a strong inhibition of the acceptor side of PSI occurs including a possible reduction in the size of the PSII antennae [35].

As shown in Supplementary materials, Fm and F0 values depended on the density (biomass) of the culture expressed as OD_{750}. The F0 parameter values were statistically similar in the OD_{750} range from 0.12 to 0.50, while the Fm values increased rapidly with an increase in the OD_{750} value (see Supplementary materials - Fig. 2A and B). In this context, the higher F0 values observed in the cultures after 8 days could be associated with a significant increase in biomass when compared with the earlier growth periods (Fig. 2C and I). The exception was the *C. emersonii* culture, characterized by a weak growth dynamic where F0 value changes in the study period were low (Fig. 2D–F). However, on successive days of algae culture growth differences in the F0 value (point o on the OJIP curve) were observed, depending on the spectral composition of light (Figs. 2 and 3). Higher F0 values are indicative of a physical separation of PSII from its pigment antenna and, consequently, an inhibition of electron transfer to the reductant side of PSII [33]. Furthermore, the increase in F0 phosphorylation is attributed to the PSII light-harvesting complex (LHCII), resulting in the dissociation of LHC from PSII in order to protect plants from excessive light intensity [36]. The study by Tóth et al. [37] also demonstrated that an increase in F0 was associated with a greater pooling of reduced plastoquinone (PQ), which induced LHCII phosphorylation [36]. LHCII are important for an optimal photosynthesis process, as they are responsible for the redistribution of energy between PSI and PSII.

On the basis of the JIP-test and PSII photosynthetic parameters, it can be concluded that the spectral composition of light affected the acclimatization of different strains of algae to the environment and to an increase in biomass in different ways. With regards to *C. vulgaris*, the functioning of the photosynthetic apparatus was most optimal under WL, while it was most optimal in *C. emersonii* and *B. braunii* under B-R-FRL. The above data and the data obtained previously [28] suggests that the beneficial effect of B-R-FRL and WL on the photosynthetic apparatus could be due to the presence of FRL in both spectra.

The differences described between each day of cultivation (days 2, 4, and 8) in the fluorescence intensity of cells of the algal strains under study in the R-FR range probably resulted from the different phases of growth of these cultures. Thus, the *C. vulgaris* and *B. braunii* cultures on day 8 were in the logarithmic growth phase, while *C. emersonii* was still in the lag phase.

Measuring the fluorescence of individual PS components resulting from the excitation of chlorophyll molecules is an effective way to assess the impact of abiotic and biotic stress factors on photosynthetic activity [38]. PSI and PSII emissions in *C. vulgaris* and *C. emersonii* were dependent upon both the spectral composition of light and the stage of growth. However, in the case of *B. braunii* the effect of the spectral composition of light, with respect to PSII A/C and PSI/PSII ratios (Table 1, Fig. 4), was observed only on day 8. The higher values of the PSI/PSII ratio observed in *C. vulgaris* and *B. braunii* on day 8, when compared with that on days 2 and 4, showed an inhibition of PSII after 8 days of growth (Table 1). This may suggest a decline of the logarithmic growth phase in these algae. Highly varied, depending on the color of light and day of growth, the PS fluorescence parameters obtained for *C. emersonii* showed that the cultures in the lag phase of growth were strongly influenced by factors like the color of light and the age of a culture. The impact of these factors on PS in cultures already in the logarithmic growth phase was much less pronounced (*C. vulgaris* and *B. braunii* – day 8; Table 1). The values of the PSII A/C ratio were significantly higher on the 2nd and 4th days than on the 8th, which showed that in the early stages of growth, *B. braunii* and *C. vulgaris* exhibited a greater reduction in fluorescence emission in PSII C than in PSII A. In turn, on the 8th day the higher values of PSII A/C in the *C. vulgaris* species of algae observed under B-RL than under WL showed how this color of light affected the energy transfer between PSII A-C (Table 1). In contrast, the constant PSII A/C ratio in *C. emersonii* growing under B-R-FRL showed that the A-C energy transfer remained the same under the conditions of this spectral composition of light during the period of growth under analysis.

Disclosures

The authors have no conflicts of interest to declare.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jphotobiol.2017.01.013.

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