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Assessment of phosphorescent paint effects on microalgae cultivation

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Abstract−Light is the most important factor involved in the growth of photosynthetic organisms, and low efficiency of artificial light systems imposes higher cultivation costs. Phosphorescent paints can emit light for a few hours, and so may be appropriate to accumulate scattered light. In this study, the effects of blue and green phosphorescent paints on growth rate, biomass production and chlorophyll content of three different microalgae, *Scenedesmus dimorphus*, *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were investigated. These strains were cultivated in broth medium in three glass bowls. Half of the first two bowls was stained with blue or green phosphorescent paints (B and G treatment), while the third one was unstained and used as the control (C treatment). All measured parameters were higher in B. The results indicate that partial staining of culturing bowls by blue phosphorescent paint can be an economic approach to increase light efficiency in cultivating microalgae.

Keywords: Illumination Efficiency, Microalgal Cultivation, Persistent Luminescence Paint, Chlorophyll Content

INTRODUCTION

Microalgae are among the photosynthetic organisms used in production of biofuels, food coloring, animal feed, bio-fertilizers, etc. [1]. The growth and chemical composition of microalgae is affected by both environmental and biological factors which include temperature, pH, light irradiance, presence of other microorganisms and incubation cell density. Light is one of the most important environmental factors influencing the growth of microalgae through photosynthesis [2]. Since the rate of photosynthesis would decrease in the absence of light, artificial light is being applied to drive photosynthesis, and so becomes the most expensive factor affecting the microalgae growth [3,4].

The two most commonly used methods for cultivation of microalgae are open cultivation systems (open ponds, raceway ponds and tanks) and closed cultivation systems using photo-bioreactors (PBRs). Since the first attempts using open raceway ponds [5], extensive research has been carried out to improve microalgae cultivation in open systems. Major advantages of open systems are minimal costs and energy requirements. However, they allow restricted or no control over growth parameters such as temperature and evaporation, require large areas and are susceptible to contamination and climate changes [6,7]. Climate change in the form of diurnal and seasonal changes also comes into play as they lead to varying light irradiance and photoperiods. In contrast, PBRs use relatively little space while providing minimal risk of contamination. Furthermore, PBRs offer better control on culturing conditions such as nutrient supply, temperature, pH, mixing rates and exposure to light [8]. These advantages nominate PBRs as a better choice over open systems. However, these systems also have their own disadvantages, including bio-fouling, benthic algae growth and cleaning issues, and more importantly, very high setup and operational costs [9,10]. Artificial illumination can be applied to PBRs while light sources can even be placed inside the reactor to increase the light availability [11,12].

Both systems however, are inefficient with respect to light consumption. One approach to increase light availability to microalgae is to increase the surface-to-volume ratio. This requires devoting a large area to open systems and so increases the capital costs. However, increasing the illumination efficiency would reduce the production costs and enhance microalgae growth and yield [13].

Luminescence is a phenomenon released as energy in the form of light, in response to an excitation input in the form of light, heat, pressure or any other means. Luminescent material exhibit such phenomenon that can be categorized as fluorescence or persistent luminescence, based on the time taken to produce output [14]. In fluorescent materials, commonly named phosphors, light absorption brings the luminescent impurity into an excited state, with a typical lifetime of nanoseconds up to a few milliseconds. Persistent luminescence, named long-lasting phosphorescence, refers to the phenomenon where a luminescent material continues emitting light even when the excitation source is no longer active. Several terms like "phosphorescence," "persistent phosphor," "long-lasting," "persistent," "afterglow," and any combinations thereof are used to explain this process. In persistent luminescent materials, energy is stored in traps, and thermal energy (even room temperature) is required to release the trapped charge carriers within an average period of seconds to hours, leading to the delayed light emission

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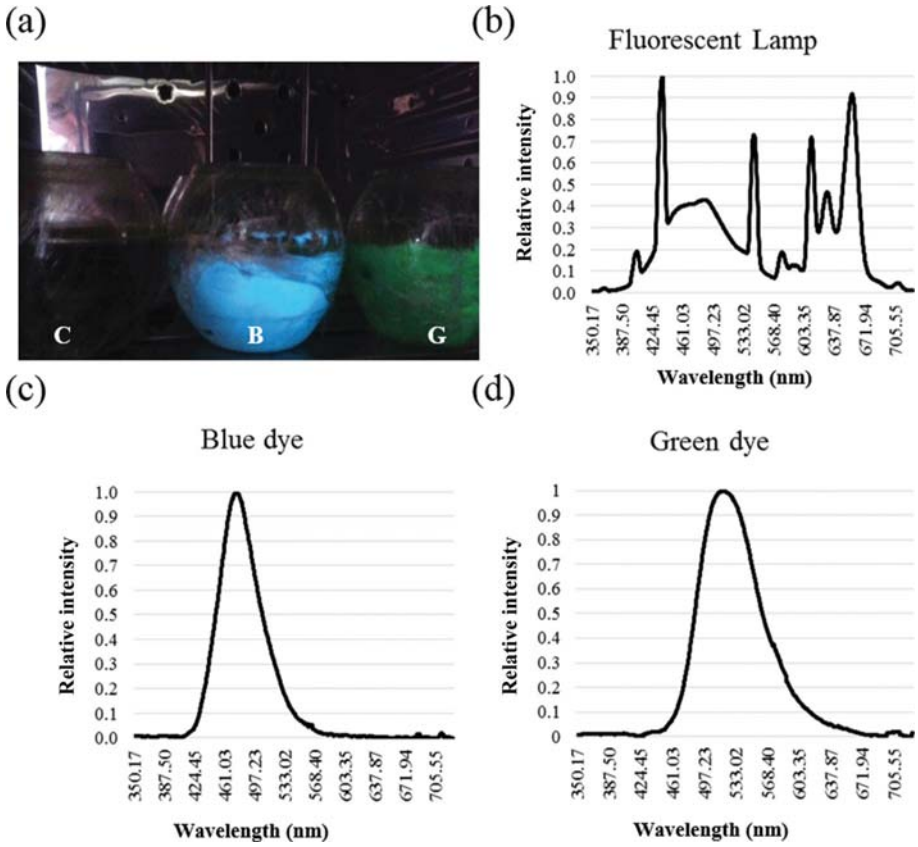

Fig. 1. (a) Side view of glass bowls after excitation; control (C), blue (B) and green (G) phosphorescent paints. (b) Optical spectrum of fluorescent lamps which function as photosynthetic light source and excite the phosphorescent pigments in B and G. (c) Emission spectrum of B. (d) Emission spectrum of G.

[15,16].

Phosphorescent paints, mostly made of Strontium Aluminate (e.g., doped with Europium Dysprosium oxides, SrAl2O4.Eu,Dy), are capable of emitting light for tens of hours after ten minutes to one hour excitation. They are made from rare earth phosphorescent pigments, and have numerous applications especially after the mid-1990's discovery of a new class of very efficient compounds [17]. Since then, the number of materials with persistent luminescence features has continued to increase, leading to more than 250 materials covering a wide range of emission wavelengths [18].

This study, for the first time, evaluates the growth of microalgae illuminated by the afterglow of luminescent paints as complementary light source. Phosphorescent pigments of the paint become excited by a fraction of photosynthetic light not absorbed by the microalgae, and illuminate the culture with the afterglow. Three strains of microalgae, *Scenedesmus dimorphus*, *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, were used in this trial.

METHOD AND MATERIALS

S. dimorphus (Clean Nature Explorers Co.), *C. reinhardtii* (Strain 137c, Chlamydomonas resource center, university of Minnesota), *C. vulgaris* were cultivated in BBM [19], TAP [20] and Z8 [21] media, respectively. Prior to inoculation, each strain was cultivated for ten days in a 1 L bowl placed on a rotary shaker with 120 rpm inside a growth chamber. The temperature was set to 25±0.5 °C. Photosynthetic light was provided with six white fluorescent tubes (NARVA Fleurlight, NARVA Lichtquellen GmbH+Co. KG, Brand-Erbisdorf, Germany) with 16/8 h light/dark cycles. Light intensity was 96 μmol m^{-2} s^{-1} above the bowls. Three independent glass bowls (1 L each) were prepared for each strain. Except for the control, the outer surface of two of the three bowls was stained with green and blue phosphorescent paints. As shown in Fig. 1, staining of each bowl covered half its height so that photosynthetic light could reach the medium. The optical spectrum of fluorescent lamps as well as phosphorescent paints was measured by using an Ocean Optics USB 2000 Spectrometer (Fig. 1(b)-(d)).

Each strain was inoculated in each of the three bowls. At the time of inoculation, the inoculum cell count, count after inoculation, inoculum age, pH and chlorophyll *a* content were measured (Table 1). Growth continued under the same conditions as before. To further exclude positional effects on growth, the bowls on the shaker were replaced every five days. Growth performance was monitored by counting the number of cells (by hemocytometer). Samples were taken from each bowl to measure chlorophyll content. They were extracted in 80% acetone, and the absorptions were measured in 412, 431, 460 and 480 nm (according to [22]). For biomass measurement, three samples each containing 35 ml of the culture medium in each bowl were taken. The specific growth rate (Day^{-1}) for each strain/treatment was calculated using the equation:

Table 1. Summary of inoculation conditions

	Chlorella vulgaris	*Chlamydomonas reinhardtii*	*Scenedesmus dimorphus*
pH of inoculum	10.62	8.38	11.08
Count of inoculum	1×10^8	$2.35\text{-}2.5\times10^6$	$\sim1\times10^6$
Count after inoculation*	$\sim1\times10^6$	$\sim1\times10^6$	$\sim1\times10^6$
Inoculum age	11 days	10 days	10 days
Culture volume of each flask (mL)	800	850	800
Chlorophyll *a* content of Inoculum (μg/mL)	7.427	10.47	2.85

*According to [4]

$$\mu=\ln (N_t/N_0)/T_t-T_0$$

in which N_0 and N_t are the number of cells at the start and end of log phase, respectively. T_t and T_0 indicate the days on which the log phase started and ended (according to [23]).

To monitor the biomass changes, the dry matter of two test samples, each containing 35 mL, was measured. Statistical analysis was performed on experimental values (one-way ANOVA, $p<0.05$ [24]).

RESULTS

Growth parameters measured for *S. dimorphus* are presented in Fig. 2. From day 6, the number of cells within the blue bowl (B) exceeded that of the other treatments (Fig. 2(a)). The trend continued throughout the rest of the experiment and by day 12, the cell count in this treatment became 27% more than that of the control sample (C). The cell count in green phosphorescence treatment (G) exhibited lower values compared to the control sample.

Biomass on day 6 was not considerably different in all treatments. On days 13 and 18, biomass in G was almost equal to that of C, but higher values were recorded for cells grown in B (70 mg/L and 210 mg/L higher than C on days 13 and 18, Fig. 2(b)).

Chlorophyll *a* and *b* content recorded for B exceeded that of the other treatments from day 4 (Fig. 2(c) and (d)). In contrast to the superiority of B, there was no clear distinction between the content of chlorophyll *a* and *b* in treatments G and C. However, the lowest chlorophyll amount, especially in case of chlorophyll *a*, was detected in treatment G.

Chlamydomonas reinhardtii in G presented lower cell count compared to C from day 4 (Fig. 3(a)). The number of cells in B exceeded

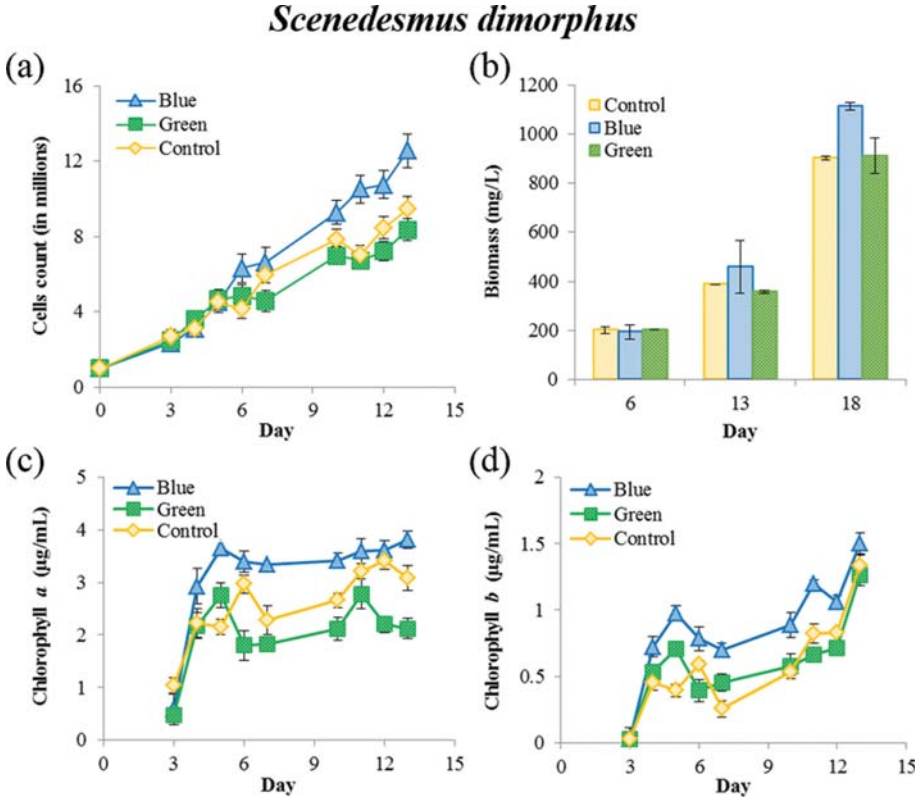

Fig. 2. Growth behavior of *Scenedesmus dimorphus* grown in control (C), blue (B) and green (G) treatments. (a) Cells count. (b) Dry biomass. (c) Chlorophyll *a* content. (d) Chlorophyll *b* content.

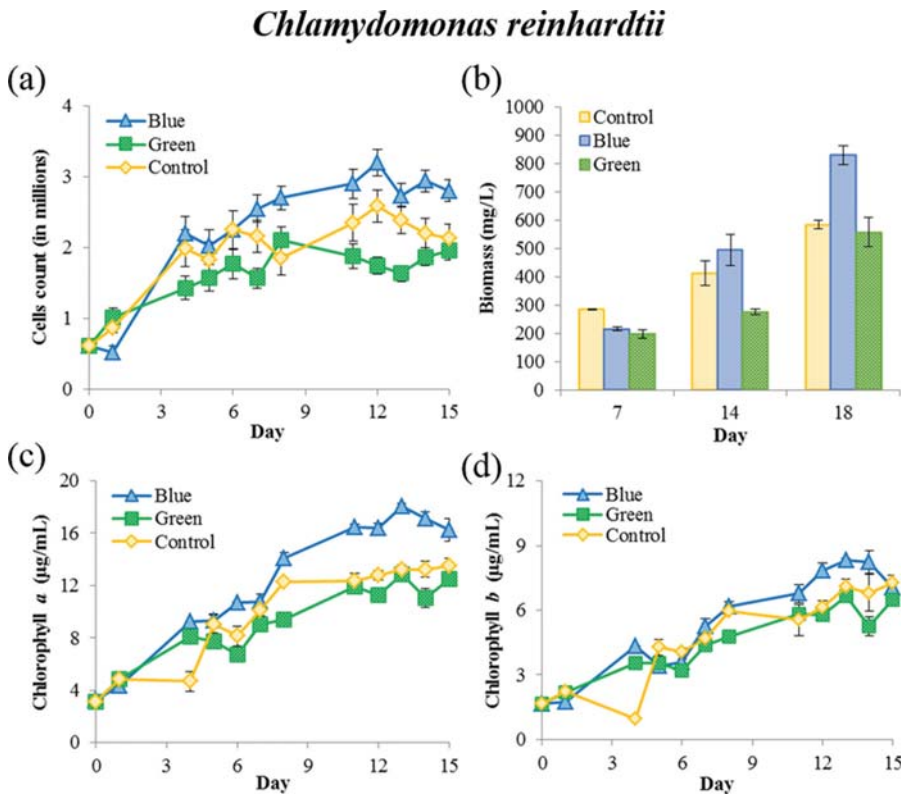

Fig. 3. Growth behavior of *Chlamydomonas reinhardtii* grown in control (C), blue (B) and green (G) treatments. (a) Cells count. (b) Dry biomass. (c) Chlorophyll *a* content. (d) Chlorophyll *b* content.

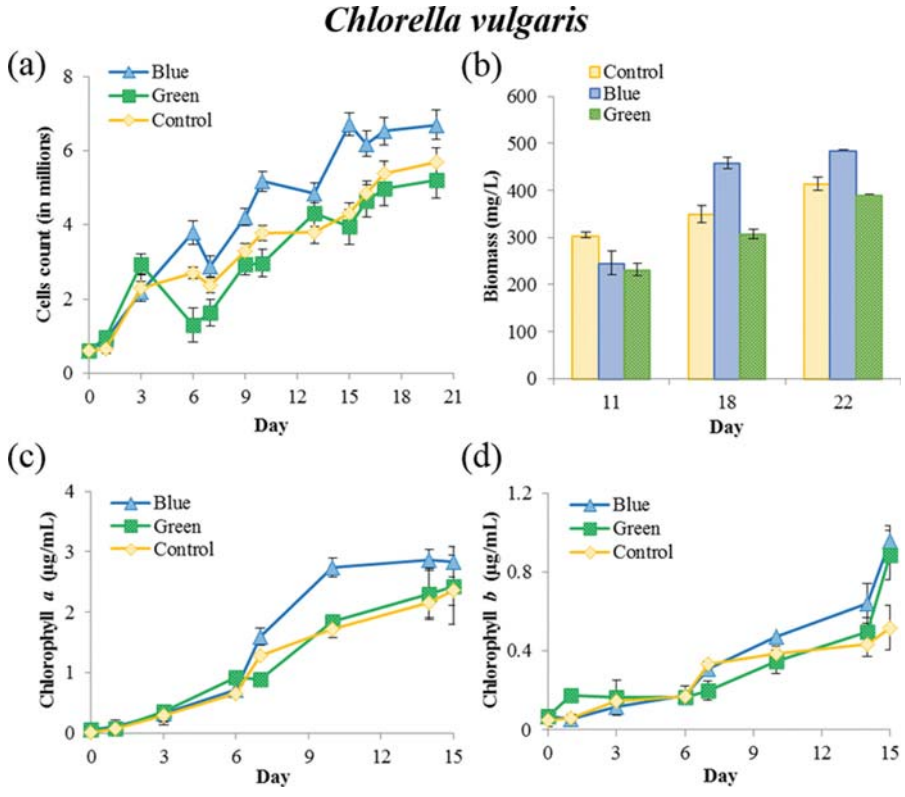

Fig. 4. Growth behavior of *Chlorella vulgaris* grown in control (C), blue (B) and green (G) treatments. (a) Cells count. (b) Dry biomass. (c) Chlorophyll *a* content. (d) Chlorophyll *b* content.

other treatments from day 7. The increase in the number of cells ceased or reversed from day 12. At the end of the logarithmic growth phase, the number of cells in B was 23% higher than that of C.

With respect to biomass, highest values were recorded initially for C (day 7, Fig. 3(b)). On day 14, however, cells grown in B achieved maximum biomass, while those in G stayed as the minimum. This trend continued for day 18. Compared to C, biomass in B on days 14 and 18 was 82.85 mg/L and 245.72 mg/L higher in B, respectively.

Highest chlorophyll *a* and *b* content of *C. reinhardtii* was seen in treatment B (Fig. 3(c) and (d)), in parallel with the other strains. While chlorophyll *a* content in treatment B was clearly separated from the amount measured for the other treatments from day 8 (Fig. 3(c)), chlorophyll *b* content did not present big difference among treatments (Fig. 3(d)). The biggest difference in chlorophyll content, more clearly presented in chlorophyll *a*, was detected on day 13. Although more pronounced for chlorophyll *a*, the lowest chlorophyll content was detected in treatment G (Fig. 3(c) and (d)).

Chlorella vulgaris cell count in B exceeded that of C from day 6 and stayed as the maximum for the next two weeks of the experiment (Fig. 4(a)). Meanwhile, cells in G presented the minimum counts. At the end of logarithmic growth phase on day 15, *C. vulgaris* cells count in B was 55% higher than that of the control.

The pattern of changes in *C. vulgaris* biomass (Fig. 4(b)) resembled that of *C. reinhardtii*. While the highest biomass on day 11 was recorded for C, enhanced growth performance of cells cultured in B enabled them to achieve the maximum biomass values on days 18 and 22 (108.57 mg/L and 71.43 mg/L higher than the control treatment on the same days, Fig. 4(b)). For all measurements, minimum biomass values were recorded for G.

Chlorophyll content in *C. vulgaris* was not significantly affected during the initial seven days of the treatments (Fig. 4(c) and (d)). From day 7, however, chlorophyll *a* content in B exceeded that of G and C and remained superior throughout the rest of the experiment (Fig. 4(c)). Accordingly, maximum chlorophyll *b* content was also recorded for B, although it became evident from day 11 and the difference between treatments was not as pronounced as chlorophyll *a* content (Fig. 4(d)). Minimum values were recorded for G. In parallel to results obtained from *S. dimorphus*, and in contrast to G, B provided a good support for the growth of *C. reinhardtii* throughout the course of the experiment.

More pronounced in case of chlorophyll *a*, cells in B presented the highest chlorophyll content from day 8 (Fig. 4(c) and (d)). Throughout the course of experiment, chlorophyll content in G was comparable to that of C.

Table 2. Analysis of variance of phosphorescent paints and strain factors on biomass, cell count, chlorophyll *a* and *b*

	P_{value}		
Response	*S. Dimorphus*	*C. reinhardtii*	*C. vulgaris*
Biomass	0.002	0.066	0.037
Cell count	0.060	0.189	0.001
Chlorophyll *a*	0.033	0.004	0.052
Chlorophyll *b*	0.017	0.068	0.001

Table 3. Specific growth rate in different treatments

	Specific growth rate (Day^{-1})		
	Control	Blue	Green
Scenedesmus dimorphus	0.154	0.174	0.146
Chlamydomonas reinhardtii	0.12	0.138	0.103
Chlorella vulgaris	0.129	0.158	0.123

According to ANOVA results, some marginal P_{value}s were obtained in the treatments of *C. reinhardtii*, but the effects of treatment on cell counts, biomass and chlorophylls were statistically significant in most cases (Table 2). The specific growth rate for the strains in C, B and G treatments is presented in Table 3.

DISCUSSION

According to the cell counts, treatment B revealed significant increase in specific growth rate during log phase, while microalgae cultivated in G presented lower specific growth rate values compared to the control. Highest biomass production was also recorded in B, whereas biomass in G was totally lower than C. The specific growth rate for *S. dimorphus* was reported as 0.113-0.148 [23,25]. Those studies have grown *S. dimorphus* under 16/8 h light/dark cycles, with 2,500-3,500 lux illumination and temperature of 23-25 °C. The reported specific growth rates however, are close to the time detected in this experiment. The specific growth rate for *C. vulgaris* illuminated with 12 : 12 h light : dark period by full spectrum xenon lamps using luminescent sheets as light filters is reported as 0.06/day [26]. Although the specific growth rate was calculated based on biomass concentration in that study, different illumination time led to different specific growth rates. This is what has been reported in the current trial of 12 : 12 h vs. 16 : 8 h illumination times [27,28].

Illumination is the most important factor in growth of microalgae in PBRs. All treatments in this study were illuminated with fluorescent lamps for 16 hours during each diurnal cycle. In contrast to the control treatment, cells cultivated in B and G received blue and green afterglow, respectively. Being more accessible, blue and green phosphorescent paints were used as a low cost means of extending the illumination period with no further energy consumption. To avoid shading the growth media within the bowls, only the lower half of each bowl was painted. In this respect, the phosphorescent paint will get excited with photons passing through the medium (not used directly by microalgae), as well as those reaching the outer surface of the bowl from outside. For all strains studied, highest growth parameters were recorded for B. It is shown that blue illumination affects the growth of *Chlorella kessleri* through increasing the cell size [4]. In parallel to specific growth rate and biomass values detected in this trial, highest specific growth rate and dry cell weight for *Nannochloropsis salina*, *N. oceanica*, and *N. oculata* are reported in blue (645 nm) illumination, while the lowest values are detected in green (520 nm) lighting [29]. In case of *Chlorella kessleri* higher biomass weight is reported for blue illumination when compared with fluorescent lamps [4]. Furthermore, several reports indicate that blue illumination leads to higher biomass in photosynthetic organisms [3,30].

In PBRs, the light duration and irradiance also affect the growth of microalgae [31]. However, different types of algae may require different optimum photon flux density and duration [32]. For example, highest biomass production and specific growth rate for *Nannochloropsis* is reported for 100 μmol/m²/s of both green and blue illumination [29], while higher light irradiances inhibit its growth. Furthermore, high light irradiances may lead to excessive temperatures, which in turn can inhibit growth and production and may even cause cell damage [33]. It is reported that continuous illumination of a PBR with 12 : 12 h photoperiod leads to less than 30% increase in biomass generation [34], despite causing higher energy costs in doubling the illumination time. It was mentioned that poor nutrient uptake achieved during dark period [34]. Introducing phosphorescent paints in this trial, however, biomass recorded in treatment B for *Scenedesmus*, *Chlamydomonas* and *Chlorella* was 23%, 42% and 17% higher than the control, with no additional energy costs for illumination. This may nominate the application of phosphorescent material as supplementary light sources in cultivation of microalgae.

Chlorophyll plays a central role in microalgae and plant photosynthesis, owing to its light absorbing feature. It is also commercially used as food coloring. Chlorophyll content of microalgae is widely being used in production of food additives [35]. Chlorophyll *a* and *b* are the two major chlorophylls that differ in molecular structure and have maximum absorption in different wavelengths. While chlorophyll *a* is a blue/green pigment, chlorophyll *b* is green/yellow [36]. Experiments performed to study the effect of blue light on chlorophyll content indicate an increase in the amount of chlorophylls *a* and *b* [37,38]. In parallel to chlorophyll *a* content, increasing photosynthetic activity is also reported under blue light [38]. Sanchez-Saavedra and Voltolina (1994) also reported an increase in chlorophyll *a* content under blue light [39]. Results obtained in the current trial indicate that blue and green phosphorescence can affect chlorophyll content in microalgae; while blue phosphorescence led to strong increase in chlorophyll *a* and *b* content, green phosphorescence mostly caused fewer chlorophyll levels. Considering the absorption spectra of chlorophyll *a* and *b* (662 and 642 nm, respectively), painting glass bowl with green phosphorescent paint in this experiment might have limited the illumination yield during light phase while not being able to promote chlorophyll increase during the dark period. Considering the high costs of chlorophyll production at industrial scale [36], phosphorescent material can increase growth efficiency and thus reduce the final price of extracted products.

CONCLUSION

Three different strains of microalgae were cultivated in glass bowls. The outer surface of two bowls was painted with blue (B) and green (G) phosphorescent paints, and the growth of microalgae in these bowls was compared with the control sample (C, not painted). Although no significant change was recorded for growth parameters in treatment G, the blue afterglow in treatment B affected the growth parameters of the three microalga strains investigated (*S. dimorphus*, *C. reinhardtii* and *C. vulgaris*). Employing the afterglow of phosphorescent materials would be an advantage in large-scale production with lower costs since it imposes no additional energy costs, but provides higher biomass, cell counts and chlorophyll content.

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