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Research article

PHYSIOLOGICAL AND GROWTH PERFORMANCE OF CYANOBACTERIUM, *PHORMIDIUM FRAGILE* UNDER EXPOSURE TO DIFFERENT LIGHT CONDITIONS

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ABSTRACT: Light is the most important environmental factor that critically affects growth and development of algae through its immediate influence on photosynthesis. In such a process, the initial phase (light reactions) involves light absorption resulting in the formation of intermediate energy compounds (ATP and NADPH), which would later be utilized for driving the second phase of photosynthesis (carbon fixation) in the Calvin cycle. In the current investigation the principal objective was to evaluate the effect of different light conditions on the physiological and growth behaviour of the Cyanobacterium, *Phormidium fragile*. To realize that goal carbohydrates content as well as dry mass production were analyzed and determined. *Phormidium fragile* tissues were propagated in sterilized flasks containing an appropriate algal culture and subjected to four different light conditions: sun light, laboratory light, white lamb light (control) and darkness. Analysis of carbohydrates content showed that the algal growth under both laboratory and darkness conditions exhibited significantly higher values (106 mg and 81 mg, respectively) relative to control (38 mg). On the other hand, the dry matter content was significantly lower in darkness and laboratory conditions (30 and 35 mg, respectively) compared to that of the control (75 mg). Based on these findings our present study provided additional supporting evidence for the significance of light energy as a determinant factor in the photosynthetic activity of algae in relation to dry mass accumulation and carbohydrate metabolism. Further studies need to be carried out in order to validate our conclusions.

Key words: *Phormidium fragile*, photosynthesis, light, carbohydrate, dry mass.

INTRODUCTION

Today world is threatened by serious environmental problems such as over- release of CO_2 in the atmosphere leading to global warming. In consequence, recent researches are trying to find out practical solutions to address this environmental hazard through the application of eco-friendly and economical renewable energy sources, such as; solar energy, wind and biofuels [1]. In this respect, algae could be a promising candidate as an alternative energy source for the great possibilities to extract biodiesel from their biomass, which does not increase the CO_2 amount in the atmosphere when burned compared to the conventional energy sources like fossil fuels [2]..

Compared to crops, algae are uniquely generally characterized by a much higher efficiency in converting solar energy to produce biodiesel, which is mainly attributed to their very low demand for water and less area for cultivation in both in- and out-door systems [3]. Until now, information available on the productivity of microalgae in growth systems is rather poor. Therefore, the current primary focus of most research subjects is the scaling up of microalgae growth systems to the commercial level of microalgae-based biofuels [4,5]. The Cyanobacterium, *Phormidium fragile* used in our current investigation belongs to Cyanobacteria (blue-green algae), which are also considered as rich sources of structurally novel and biologically active metabolites, most of which are accumulated in the cyanobacterial biomass. Recently, and apart from other usages there has been a growing interest in Cyanobacteria in general for their great potential to produce pharmaceuticals [6,7].

In general, the autotrophic organisms of which *Phormidium fragile* is no exception perform photosynthesis triggered by the availability of a set of influencing factors. The main factors affecting the photosynthetic productivity of algae include light intensity, temperature, photosynthetic rate, transpiration rate, nutrient availability and lipid production [8,9]. Principally, the requirement for light (duration and intensity) is essential for growth and photosynthesis of algae as documented in several studies [3,10,11,12]. For productive photosynthesis, a microalga requires a light/dark regime. Light is required for a photochemical phase to produce energy (ATP, NADPH) and dark is equally important for the biochemical phase to synthesize essential molecules (carbohydrate, protein and fatty acids) for growth and development [13,14,15]. In areas receiving high solar light radiation such as the tropics, development of photo-bioreactors has been in progress as a potential technology for CO_2 sequestration as reported in numerous studies [16,17,18,19]. Some studies showed species-specific variation among the algal species with respect to their efficiency in utilizing CO_2 [20,21]. In a recent study, it was well demonstrated that the duration of the photoperiod was a determinant factor in the performance of photo bioreactors in which reductions in photoperiod were observed to be coupled with reductions in biomass and carbon fixation [22].
Despite the potentially limiting effect of light energy on the growth capacity of algae to increase the biomass production, however still there are limited published data now available on Cyanobacteria especially *Phormidium fragile* and their response to variation in light conditions. Hence, the current investigation was undertaken and the main purpose was to evaluate the growth performance of a Cyanobacterium, (*Phormidium fragile)* under four variable light conditions in relation to dry mass production and the yield of carbohydrate content.

MATERIALS AND METHODS

Source of organism

Phormidium fragile was provided from King Saud University, Faculty of Science, Department of Botany and Microbiology.

Culture medium preparation and growth conditions

Pharmidium fragile was cultivated in a medium prepared according to [23] containing the following constituents (g/l distilled water): 1.5 KNO3; 0.5 K2HPO4; 0.2 MgSO4; 1.8 NaHCO3; 1.0 NaCl,; 0.059 NaCla,; 3ml EDTA, and 1 ml trace elements. A volume of 45 ml from the prepared culture at a temperature of 25°C was taken and placed in a sterilized flask under sterilized conditions. Algal culture was thoroughly gently mixed using an electric mixer to facilitate cutting the algal tissues into small pieces, the trichome, and subsequently 5 ml of the trichome were taken and transferred into the flasks containing the prepared medium. This process was replicated sixteen times ending in sixteen separate sterilized flasks.

Light treatments and culture conditions

For the application of light treatments, the flasks of algal culture were incubated for seven days and subjected to a wide range of photoperiods. Four different sources of light radiation were selected as follows: direct sun light, laboratory light, darkness and lamb light (control). Four flasks were used as replicates for each treatment (light condition).

Determination of dry mass

For dry mass determination, a known volume of algal suspension was filtered through pre-dried and pre-weighed Whatmann filter papers. The biomass on top of the filter paper was washed filtering using distilled water. The filter paper with biomass was dried at 105°C for 4 hours and allowed to cool in desiccators. Afterwards the filter paper was weighed again to obtain the dry mass of the algal cells.

Estimation of carbohydrate content

Carbohydrates content of the algal cells was determined after the methods of [24]. Five mg dry mass of the algal cells was placed in a sterilized test tube to which 10 ml of 5% TCA was added. The mixture in each test tube was incubated in a water bath (at 80 - 90°C for one hour) under frequently gentle shaking. The test tubes were allowed to cool down and from each 1 ml was taken and transferred to another sterilized tube to which 5% phenol was added with vigorous shaking. For each tube 5 ml of concentrated sulfuric acid were added and quickly mixed. The tubes were allowed to cool down for half an hour; the optical density was measured at a wave length of 490 nm. The concentration of each sample was determined from the standard curve values then the percentage of carbohydrates was quantified.

Statistical analysis

Data were statistically treated with a statistical programme JMP 5.1 Start Statistics, third edition (SAS Institute, Inc., Cary, North Carolina, USA). The variations among the different light conditions (treatments) were tested using One-Way-ANOVA. The results presented are means (4 replicates ± SE). Separation of means was performed by Tukey – test. A probability level of $P \leq 0.05$ was considered to indicate significant differences.

RESULTS AND DISCUSSION

Dry mass production

Statistical analysis of data showed that the dry mass of the Cyanobacterium, *Phormidium fragile* cells was significantly higher in both control (white light) and sun light compared to the other treatments; lab light and darkness (Figure 1). These findings are in agreement with [22] who documented remarkable decreases in biomass production with the reduction of light duration. Variations also exist among algal species in response to fluctuating light conditions in the environment and some algal species demonstrate differential abilities for acclimation and/or adaptation to these changes [25]. Since biomass yield is in proportion with the photosynthetic efficiency, recent investigations have proposed down-regulation expression of light-harvesting complexes to minimize photo-oxidative damage under extremely high light intensities with a consequent remarkable improvement in algal productivity [17,26,27].

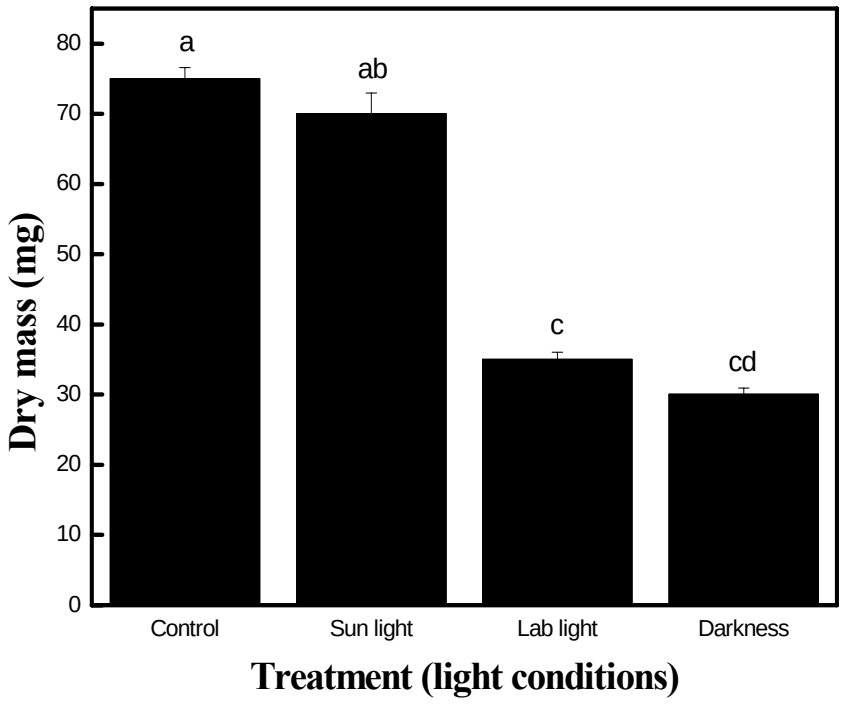

Figure (1): Effect of different light conditions on the dry mass accumulation of the *Pharmidium fragile*. Values presented are means and the standard errors, SE. In each case, the mean value was determined from 4 cultures (n = 4 replicates) per treatment. The differences among the treatments relative to the control is considered statistically significant at a probability level of $P \leq 0.05$.

Carbohydrate content

Regarding carbohydrates content in the algal tissues, however we observed that the yields under both medium light and darkness conditions were significantly higher compared to those under high light and sun light conditions (Figure 2). This result might be in agreement with earlier findings in two blue-green algae, *Oscillatoria redekei* Van Goor and *Oscillatoria agardhii* Gomont, which demonstrated improved growth efficiencies under light/dark cycles compared with continuous light condition [28]. The authors provided evidence for the metabolic control in these two species as carbohydrate synthesis was reduced under long light conditions. A similar line of evidence was found in the study of [29] in green algae exposed to blue light illumination for five hours where a significant reduction in carbohydrates content was observed. The degradation of carbohydrates was attributed in part to a possibility of new proteins biosynthesis, but also might be a response to blue light action. In accordance with our findings, [30] also reported enhancement of respiration upon illumination followed by a sequence of metabolic reactions leading to starch degradation in concomitance with protein synthesis.

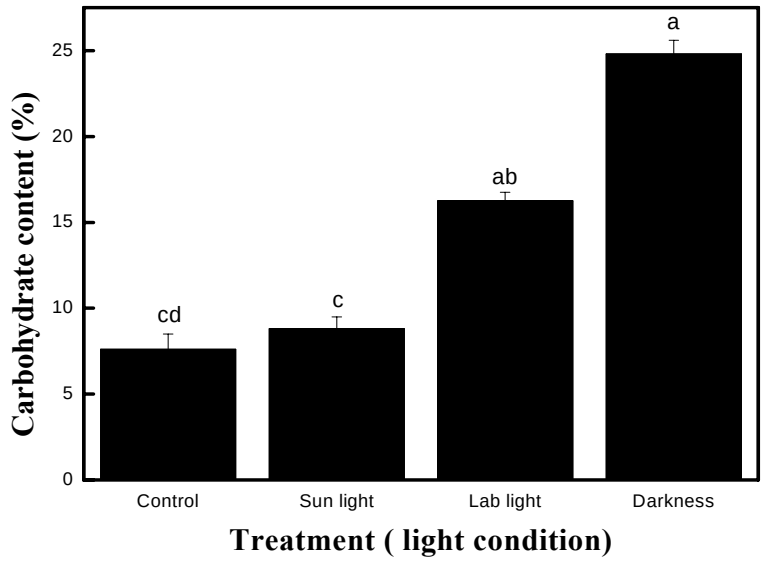

Figure (2): Effect of different light conditions on the carbohydrates content of the *Pharmidium fragile*. Values presented are means and the standard errors, SE. In each case, the mean value was determined from 4 cultures (n = 4 replicates) per treatment. The differences among the treatments relative to the controls is considered statistically significant at a probability level of $P \leq 0.05$

Since carbohydrate is needed as an energy source for respiration process in the algal cells we might expect that during continuous light condition high respiration rates are high so low level of carbohydrate compared to dark condition in which the metabolic processes are likely less active. This might lead us to a speculation that the reduced content of carbohydrate under higher level of light condition (sun light and higher light treatment) is a consequence of the greater need for carbohydrates to be utilized in order to offer energy through respiration process for the more active metabolic processes in *Pharmidium fragile* under these two light conditions. This speculation might be supported with the findings of [31] who reported that under continuous light cellular respiration of algae was higher compared with light/dark cycle.

CONCLUSION

The present work was an attempt to evaluate biomass productivity and carbon fixation potential under different light conditions for the Cyanobacterium, Phormidium fragile. In the light of our present findings it was obvious that exposure to both high and medium light conditions induced better accumulation of dry mass in parallel with a remarkable depression in carbohydrate production. Given the data in this study, it is hard and likely unwise to draw solid conclusions as net photosynthetic rate was not actually determined.

Though the current results gave only a broad image of the patterns observed, nevertheless, it might be encouraging to suggest designing and performing further experimental trials involving special combinations of light/dark cycles to optimize the yield of both targets. A challenging task is to provide more detailed information on adequate light requirements for optimal algal photosynthetic growth which would no doubt pave way for maximizing the anticipated multi products from algae.

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