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Impact of different culture conditions on growth and biopigments of *Anacystis* (*Synechococcus*) *nidulans*

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ABSTRACT

The present study makes a comparative investigation of different commercially important pigments in *A. nidulans* under different culture conditions. Temperature ranges, i e 25–29 °C and 29–36 °C were provided to the cultures under three different types of culture conditions, i e constant light, alternate light and dark condition (12:12) and natural day light condition. Cultures at temperature 29–36 °C receiving alternate light and dark period (12:12) showed maximum growth with biopigment accumulation and minimum was observed in constant light at 25–29 °C. Continuous protein synthesis in the dark at the expense of the stored carbohydrates might be the reason of high biliprotein and carotenoid synthesis which also increase its commercial and economic value. This proves alternate light and dark period (12:12) at 29–36 °C condition as a cost saving strategy for large scale production of biopigments.

Key words: *Anacystis nidulans*, Carotenoid, Culture condition, Phycobiliprotein, Temperature

Microalgae combine properties typical of higher plants (efficient oxygenic photosynthesis and simplicity of nutritional requirements) with biotechnological attributes proper of microbial cells (fast growth in liquid culture and ability to accumulate or secrete some metabolites). This particular combination supports the use of these microorganisms for applied processes. The production of food, feed and valuable products directly from algae could be a benefit to the agricultural sector because of the lower land and water quality requirements of algae ponds. Compared with the higher plants, algae have one more advantage as they can also be cultivated in bioreactors on a large scale and thus are a continuous and reliable source of the product. Marginal or erodible soils could be retired from traditional agricultural cultivation and be devoted to the growth of algae. Algolculture would complement traditional agriculture and should be welcomed as a development that will strengthen the agricultural sector. Thus, from the standpoint of agriculture the cultivation of algae for the production of marketable products is a potentially profitable venture.

The cyanobacteria, commonly known as blue-green algae, are structurally diverse assemblage of gram negative eubacteria characterized by their ability to perform oxygenic photosynthesis. In recent few decades, cyanobacteria have gained considerable importance for their possible use in food, fuel, fertilizer, production of various secondary metabolites and pollution abatement (Abed *et al.* 2009). Apart from these, they represent an attractive source of natural colours. In addition to chlorophyll, as the primary photosynthetic pigment, microalgae contain a multitude of pigments which are associated with light incidence. The pigments improve the efficiency of light energy utilization (phycobiliproteins) of photosynthetic reaction center and protect them against solar radiation (carotenoids) and related effects (Pulz and Gross 2004). Biopigments have great commercial value as natural colourants in nutraceuticals, cosmetic and pharmaceutical industry, besides their health benefits.

Anacystis nidulans is a small blue-green alga which has many of the attributes desired in an experimental organism for photosynthetic studies. For functional studies of photosynthetic pigments, we have used the cyanobacterium *Anacystis nidulans*. A useful feature of the organism is that its pigment composition can be manipulated to obtain wide variations.

On the role of pigments as light absorbers, Jones and Myers (1965) concluded that phycocyanin is the major collector of light in *A. nidulans*. Myers and Kratz (1955) reported that this pigment could account for as much as 24% of the dry weight of the cell.

The optimization of the yield is the main factor in large

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scale production of microalgae. Thus, it is necessary to understand the behavior of algal species under different environmental factors that determine the different growth parameters. Overall, the growth and biochemical composition of microalgal populations depends on many factors, the most important of which are nutrient availability, temperature and light (Carvalho and Malcata 2003, Bardhan *et al.* 2008, Samuel *et al.* 2010, Pandey *et al.* 2011). In the presence of non-limiting nutrients, the efficiency of microalgal culture remains controlled mainly by light intensity and temperature.

Blue green algae are shade-adapted organisms, possessing efficient mechanisms to counteract the harmful effects of solar radiation. Hence cyanobacteria will optimize the harvesting of light for available irradiance and spectral composition by modulating their antenna pigment composition (Grossman *et al*. 2001, De Marsac 2003). In *A. nidulans*, pigment concentrations and lamellar content vary inversely with light intensity, but the ratio of chlorophyll to phycocyanin does not vary. However, under certain growth conditions, such as a change in light quality the ratio of chlorophyll to phycocyanin does deviate from the normal. Jones and Myers (1965) showed that cells grown in red light have a reduced chlorophyll-phycocyanin ratio owing to a decrease in chlorophyll content.

The purpose of the present study is to evaluate the influence of different culture conditions of temperature and illumination on the growth and pigment content of *A. nidulans*, to optimize the culture condition for maximum bio-accumulation of pigments.

MATERIALS AND METHODS

The organism used in this study was the cyanobacteria *A. (Synechococcus) nidulans* isolated from the Mawatha Lake in Jaipur (Rajasthan, India), were grown photo-autotrophically in BG-11 medium (Stanier *et al.* 1971).

Experiment to evaluate the effect of different culture conditions on *A. nidulans* were carried out in departmental laboratory. In order to find out optimum culture condition, cultures were subjected to five different combinations of temperature range and illumination, i e Series receiving constant light at 25–29 °C (CL) (Set I), Series receiving alternate light and dark period (12:12) at 25–29 °C (AL) (Set II), Series receiving constant light at 29–36 °C (CL) (Set III), Series receiving alternate light and dark period (12:12) at 29–36 °C (AL) (Set IV), Series receiving natural day and dark period at North facing window 29–36 °C (NDL) (Set V).

Conical flasks of 500 ml capacity were prepared containing 250 ml BG-11 media and 50 ml *Anacystis* culture (inoculum). Conical flasks in triplicates were subjected to the above mentioned each of the five sets of culture conditions. Cultures were shaken gently thrice a day to avoid clumping and accelerate the growth process.

Observations were carried out over a period of five weeks after initial readings. Growth was followed through optical density (OD) and dry weight. Biomass was determined by optical density of cultures at 670 nm using Shimadzu UV/VIS spectrophotometer. The dry weight against standard absorbance unit was followed throughout the experimental period. 75-100 mL samples of culture were filtered on Whatman GF/C filters, rinsed with distilled water, and weighed after drying for 24 hr at 80 °C.

Spectrophotometer was used for pigment estimations in different cultures of *A. nidulans.* The chlorophyll-a content of samples were estimated by Parson and Strickland (1965) method, carotenoids by Jensen (1978), and phycobiliproteins by Bennett and Bogorad (1971) method.

Effect of different culture conditions on growth and biopigment accumulation of *A. nidulans* were compared by one way analysis of variance (ANOVA). Statistical analysis of data was carried out using MS Office Excel analysis ToolPak. The significance between pairs of variable means was analysed using least significant difference (LSD) test at 5% level of significance (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Biomass productivity and growth rate

Between both of the light conditions tested i e AL (Set IV) and CL (Set III) of temperature range 29–36 °C, maximum growth and biomass productivity were observed in AL 29–36 °C (Set IV), which showed an increase of about 3.77 times in dry weight and 3.5 times in OD as compared to the initial records. On the other hand, in CL 29–36 °C (Set III) the dry weight and OD were increased only about 3 times and 2.8 times respectively that of the initial readings (Fig. 1).

At temperature range 25–29 °C, cultures of AL (Set II) condition showed higher growth, where OD increased 3.17 times of the initial record, on the other hand in CL (Set I) the OD enhanced slowly and finally at the end of the Vth week an increase of 2.5 times of the initial value was observed (Fig. 1a). The dry weight results also supported this data. The dry weight at AL 25–29 °C (Set II) showed an increase of about 3.4 times of initial record while at CL 25–29 °C (Set I) the increase in dry weight was only 2.7 times that of the initial reading (Fig. 1b).

Growth of *A. nidulans* in NDL conditions at 29–36 °C (Set V) was lower as compared to other culture conditions except CL 25–29 °C (Set I). OD and dry weight were increased only 2.67 and 2.8 times respectively.

Apparently, cultures grown in AL 29–36 °C (Set IV) were healthy and dark blue green in colour while cultures under CL 25–29 °C (Set I) were pale blue green in colour. (Fig. 2).

Growth rate of *A. nidulans* was evaluated in different culture conditions for a period of initial two weeks (i e growth rate in nutrient rich medium) and for five weeks (i e overall growth rate) (Fig 3). Growth kinetics shows that for

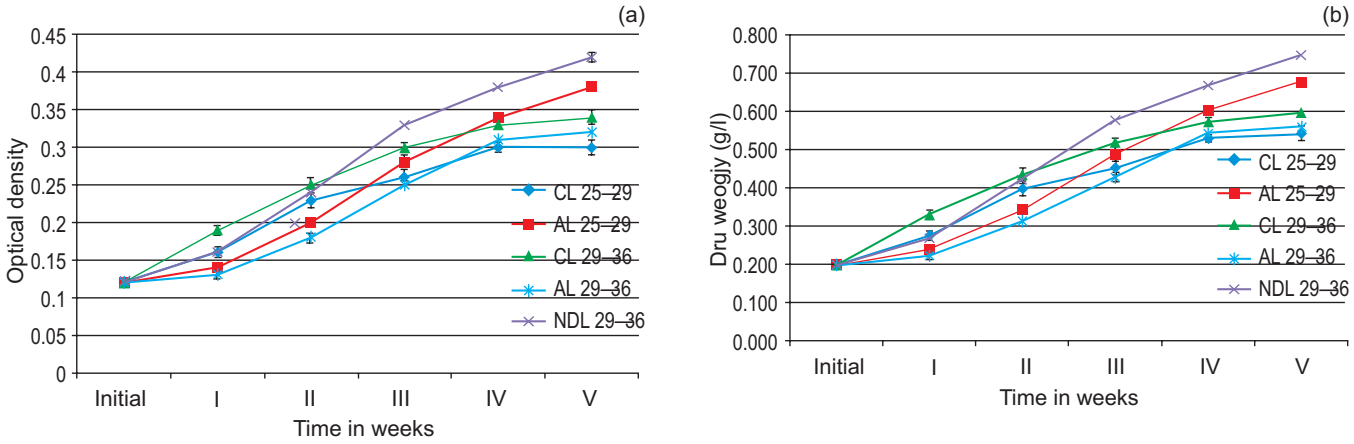

Fig 1 Impact of different culture conditions on growth of *Anacystis nidulans.* (a) Optical density, (b) Dry weight (g/l). Values are means ± SD (n=3)

Constant light at 25–29 °C (CL 25-29) (Set I); alternate light and dark period (12:12) at 25–29 °C (AL 25-29) (Set II); constant light at 29–36 °C (CL 29-36) (Set III); alternate light and dark period (12:12) at 29–36 °C (AL 29-36) (Set IV); natural day and dark period at north facing window 29–36 °C (NDL 29-36) (Set V)

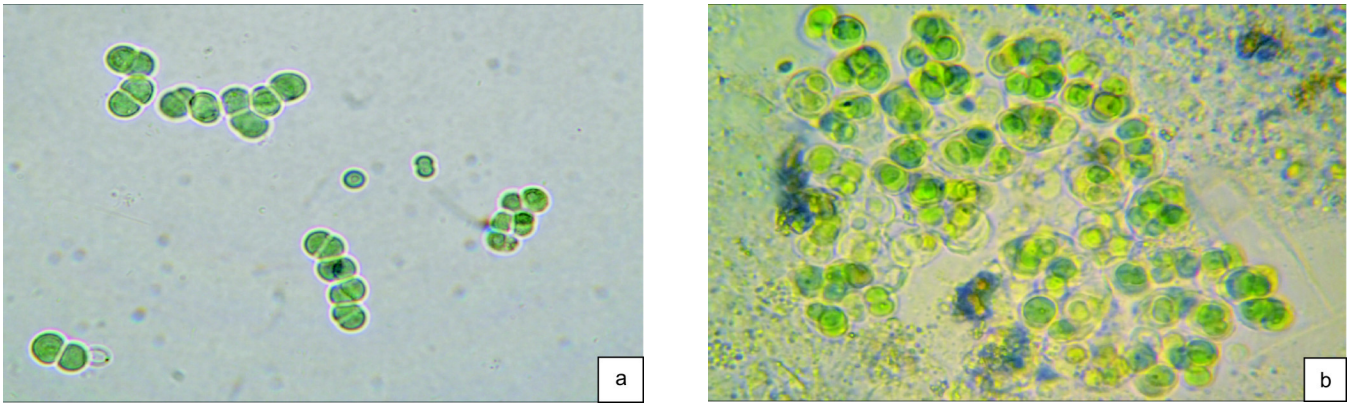

Fig 2 Cultures grown in (a) AL 29-36°C (Set IV) (b) CL 25-29°C (Set I)

initial two weeks when the medium was nutrient rich, maximum biomass production rate was found in CL 29–36 °C (Set III), followed by alternate light and dark period (12:12) at 29–36 °C (Set IV). But overall growth rate of cultures for a period of 5 weeks in the same batch of medium, shows that maximum growth rate was found in alternate light and dark period (12:12) at 29–36 °C (Set IV), followed by alternate light and dark period at 25–29 °C (Set II), constant light at 29–36 °C (Set III), north facing window receiving natural day light at 29–36 °C (Set V) and minimum was observed in constant light at 25–29 °C (Set I).

Among all of the culture conditions experimented upon, for initial two weeks maximum biomass and growth rate were found in constant light at 29–36 °C afterwards continuous illumination suppressed the synthesis of chlorophyll and other accessory pigments. This might be due to the consumption of nutrients for continuous synthesis of carbohydrates and chlorophyll, which makes the culture medium nutrient deficient for growth of *A. nidulans*. So after initial two weeks when medium was nutrient limited growth

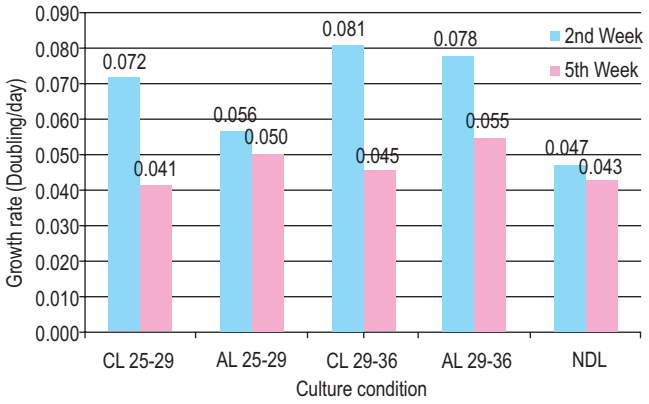

Fig 3 Growth rate of *Anacystis nidulans* in different culture conditions.

Constant light at 25–29 °C (CL 25-29) (Set I); alternate light and dark period (12:12) at 25–29 °C (AL 25-29) (Set II); constant light at 29–36 °C (CL 29-36) (Set III); alternate light and dark period (12:12) at 29–36 °C (AL 29-36) (Set IV); natural day and dark period at north facing window 29–36 °C (NDL 29-36) (Set V)

of continuous light cultures was not comparable with cultures grown in alternate light conditions. But overall maximum biomass production and pigment biosynthesis were observed in alternate light and dark period (12:12) at 29–36 °C (Set IV) and 25–29 °C (Set II) in comparison to constant light at 29–36 °C (Set III) and 25–29 °C (Set I).

Temperature is an important physical factor in the growth of algae cultures. In all of the culture conditions temperature range 29–36 °C produced higher biomass and pigments as compared to 25–29 °C temperature range. These findings also correlate with the response of cyanobacterium *Spirulina platensis* in different culture conditions (Kumar *et al.* 2008). Ranjitha and Kaushik (2005) also observed 30 °C and 35 °C temperature for highest biomass of *Nostoc muscorum*, while carotenoids and phycoerythrin were highest at 30 °C. Other workers have reported 37°C as optimum for *Arthronema africanum* (Chaneva *et al.* 2007), 36 °C for *Synechococcus* (Sakamoto and Bryant 1998), while for *Spirulina*, it is in the range 30°–38° C (Ogbonda *et al.* 2007, Rafiqul *et al*. 2003). Low temperatures not only limit the rate of photosynthesis but also make alternative ways of deexciting chlorophyll making it less efficient (Ögren *et al*. 1984).

Post *et al.* (1985) also reported maximal growth rate of *Oscillatoria agardhii* when light irradiance was supplied continuously in only turbidostat cultures. Cultures of *A. nidulans* grown in continuous light also support these finding till the media was nutrient rich. Under conditions of continuous light, the flow of carbon to all biomolecules, such as proteins, carbohydrates, nucleic acids and lipids, will occur at a rate equal to the growth rate. However, with an L/D cycle, cyanobacteria accumulate carbohydrates in the light and use them in the dark (Van Liere *et al.* 1979). This observation could explain the fact that protein synthesis was found to continue in the dark at the expense of the stored carbohydrates.

Alternate light and dark period 12:12 (at 29–36 °C and 25–29 °C) showed high growth as in comparison to constant light (at 29–36 °C and 25–29 °C). This reduction in growth was due to photooxidative death because in many algal species, prolonged exposure to high light may cause photooxidative death (Abeliovich and Shilo 1972).

Biopigment accumulation

The pigment contents of algae correlates with the growth of *A. nidulans*. The maximum pigment contents observed in the AL 29–36 °C (Set IV). Significantly higher amount of chlorophyll-a and carotenoid contents were found in cultures receiving AL 29–36 °C (Set IV), i e 1.41% and 0.097%, and followed by AL 25–29 °C (Set II), i e 1.37% and 0.093% respectively. CL 29–36 °C (Set III), NDL 29–36 °C (Set V) and CL 25–29 °C (Set I) show insignificant change in chlorophyll-a and carotenoid contents (Fig 4).

All of the cultures receiving different culture conditions show significant change in phycobiliprotein (phycocyanin, phycoerythrin, and allophycocyanin) accumulation. PBP also showed maximum bioaccumulation in cultures receiving alternate light and dark period at 29–36 °C (Set IV) that had 9.83% PC, 1.672% PE and 4.42% APC, followed by cultures receiving AL 25–29 °C (9.18% PC, 1.462% PE and 4.24% APC), constant light at 29/36 °C (8.54% PC, 1.292% PE and 4.03% APC), natural day light at 29-36 °C (Set V) (8.22% PC, 1.213% PE and 3.92% APC, and minimum was found in constant light at 25-29 °C (7.72% PC, 1.096% PE and 3.79% APC) (Table 1).

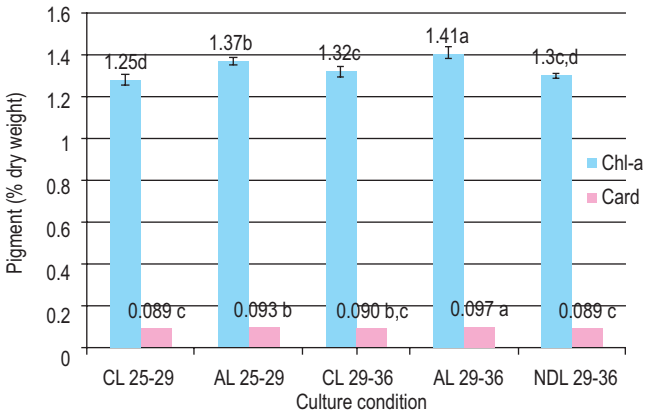

Fig 4 Effect of different culture conditions on pigments of *Anacystis nidulans*. Values are means ± SD (n=3). Variable means with the same letter are not significantly different ($P > 0.05$)

Chlorophyll-a (Chl-a), carotenoid (Cart), constant light at 25–29 °C (CL 25-29) (Set I); alternate light and dark period (12:12) at 25–29 °C (AL 25-29) (Set II); constant light at 29–36 °C (CL 29-36) (Set III); alternate light and dark period (12:12) at 29–36 °C (AL 29-36) (Set IV); natural day and dark period at north facing window 29–36 °C (NDL 29-36) (Set V)

Continuous illumination suppressed synthesis of chlorophyll. It is well documented that photoinhibition of

Table 1 Effect of different culture conditions on accessory pigments (% of dry weight) of *Anacystis nidulans*. Values are means ± SD (n=3). Variable means with the same letter are not significantly different ($P > 0.05$)

Culture condition	PC	APC	PE
CL 25-29	7.720 ± 0.046^{a}	3.790 ± 0.036^{a}	1.096 ± 0.004^{a}
AL 25-29	9.180 ± 0.035^{b}	4.240 ± 0.026^{b}	1.462 ± 0.010^{b}
CL 29-36	8.540 ± 0.026^{c}	4.030 ± 0.046^{c}	1.292 ± 0.012^{c}
AL 29-36	9.830 ± 0.056^{d}	4.420 ± 0.053^{d}	1.672 ± 0.020^{d}
NDL 29-36	8.220 ± 0.040^{e}	3.920 ± 0.046^{e}	1.213 ± 0.019^{e}

Phycocyanin (PC); phycoerythrin (PE); allophycocyanin (APC); constant light at 25–29 °C (CL 25-29) (Set I); alternate light and dark period (12:12) at 25-29 °C (AL 25-29) (Set II); Constant light at 29–36 °C (CL 29-36) (Set III); alternate light and dark period (12:12) at 29–36 °C (AL 29-36) (Set IV); natural day and dark period at north facing window 29–36 °C (NDL 29-36) (Set V)

photosynthesis in visible light may occur under conditions when the antenna of photosynthesis absorbs light in excess to what can be deexcited through photosynthesis (Powles 1984). Shuter (1979) also observed harmful impact of continuous light on chloroplast content. These findings are closely related to the contribution of Healey (1985) and Millie *et al.* (1990).

Other accessory pigments, i e carotenoids and phycobiliproteins were also found maximum in cultures receiving alternate light and dark period conditions at 29–36 °C and followed by 25–29 °C and minimum was in constant light at 25–29 °C. In alternate light condition high amount of biliproteins can accumulate due to dark cycle respiration activity in which the cells use reserve material (e g carbohydrate) for respiration. That also helps the cells to accumulate high amount of protein per volume of cell. The utilization of polyglucose in the dark was able to sustain the synthesis of all other cell components, i e carotenoids and phycobiliproteins at the same rate as when cells were growing in the light.

High amount of accessory pigments might be the reason of enhanced growth of cultures in alternate light and dark period condition at 29–36 °C, because these accessory pigments act as antenna pigments for photosynthetic light collection, and increase photosynthesis many folds. At continuous illumination and low temperature, these accessory pigments do not show progressive growth. Similar observation is also reported in the cyanobacteria *Calothrix elenkenii* by (Prassana *et al.* 2004), in *Anabaena* NCCU-9 by (Hemlata and Fatma 2009) and in red algae *Audoinella pygmaea*, *Batrachospermum delicatulum* and *Comsopogon coureuleus* (Zucchi and Neechi 2001).

SUMMARY

An experiment was conducted to evaluate the influence of different culture conditions of temperature and illumination on the growth and pigment content of *A. nidulans*, to optimize the culture condition for maximum bioaccumulation of pigments. The cyanobacterial growth and pigment estimation results revealed that *A. nidulans* showed maximum growth and pigments in alternate light and dark period (12:12) at 29–36 °C (Set IV).

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