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## **Final Report: Impact of light quality on biomass production and fatty acid composition in *Chlorella vulgaris***



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## Abstract

In this work, the green microalga *Chlorella vulgaris* was exposed to monochromatic light at six different wavelengths in order to study the effect on biomass productivity and fatty acid content. A significantly higher amount of biomass was produced in the treatments with yellow, red and white light compared with blue, green and purple light. There were also significant differences in total lipid content and fatty acid profile between the treatments. The green light regime gave the lowest concentration of lipids, but increased the concentration of polyunsaturated fatty acids. Thus it can be concluded that light quality significantly affects biomass productivity, total lipid concentration and fatty acid profile in the microalga *Chlorella vulgaris*. Also dense biofilm formation and aggregated growth of cells were observed in treatments exposed to blue, purple and white light. Less dense biofilm formation and solitary growth of cells were observed in treatments exposed to red, yellow or green light. Microalgal biofilms are of high importance in many respects, not least from an economic perspective. The result of this work indicates that light quality plays a role in biofilm formation and that blue light receptors are most probably involved.

## Background

The present report focuses the effect of light quality on growth and production of polyunsaturated fatty acid by microalgae. The improvements in LED (Light Emitting Diode) equipment in recent years have increased the interest in using this technology for various applications including cultivation of microalgae. LED equipment is capable of producing monochromatic light and thereby providing photosynthetic organisms with different light quality. Considering energy efficiency, LEDs have an advantage compared to existing lighting solutions used for cultivation (van Leperen and Trouwborst, 2008). LED light has so far mainly been studied considering biomass productivity of microalgae, and red light has been suggested as favorable for growth due to the maximal use of this wavelength in photosynthesis (Shu et al., 2012). However, considering the potential for using LED to induce production of selected metabolites or manipulate growth in microalgae less has been done.

*Chlorella vulgaris* was used as a model strain. It is a fast growing microalgae which has promise in different areas including wastewater remediation due to its high productivity under a wide range of growth conditions (Brennan and Owende, 2010). It is also a well-known species with GRAS status which has potential to enhance the nutritional value of conventional food and feed (Görs et al., 2010).

Environmental factors such as light, temperature, nutrients and CO<sub>2</sub> composition can significantly affect lipid composition of algae (Guschina and Harwood, 2006). An increase in proportion of saturated fatty acids related to unsaturated fatty acids has been shown when *C. vulgaris* are exposed to increasing light intensity (Seyfabadi et al., 2011; Khoeyi et al., 2012). However, not only the light intensity but also the light quality, the wavelength, has been shown to affect growth and metabolite production and blue light has been suggested as favorable for lipid synthesis though less biomass production was observed (Shu et al., 2012).

The intensity, duration and quality of the light available are major factors regulating growth of photosynthetic organisms and while preparing the experiments the preliminary results suggested that the growth pattern of *C. vulgaris* was affected by the light quality. For plants, a vast amount of research has been performed in this area and it is well-known that light quality plays a major signalling role in plant development (Fankhauser and Chory, 1997). However, less is known about the effects of light quality on photosynthetic microorganisms. This aspect was therefore also studied in the present study.

## Material and methods

### 2.1. Microorganism

The microalga *Chlorella vulgaris* 211/11B, obtained from CCAP, Oban, UK, was used in the experiments. The strain was routinely cultured in Z8, a standard medium for green algae (NIVA, 1976).

### 2.2. Experimental set-up

The experiments were performed in a climate chamber, where the plants received  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR during a photoperiod of 16/8 h (light/dark). The treatments involved exposure to LED light of six different colours: blue (460 nm), green (525 nm), yellow (585 nm), red (620 nm), purple (eight parts 660 nm two part 460 nm) and white (430-730 nm). The spectral irradiance of the different LED lamps was measured by a spectroradiometer (LI-COR LI1800, USA). In order to avoid the influence of other light sources, each LED treatment was enclosed in black plastic.

The temperature in the chamber was set to 20°C. The experiment was performed as batch cultures with a volume of 500 mL Z8 (NIVA, 1976). The samples were continuously aerated with air at a rate of 0.3 vvm. In the second set-up of the experiment the samples were not aerated. The start density was  $10^4$  cells  $\text{mL}^{-1}$ . On days 2, 4, 6 and 7, samples were taken and the biomass was collected through centrifugation at 3000 g for 20 min, washed twice with distilled water, lyophilised and the dry weight was recorded. All lyophilised samples were stored at -80 °C until fatty acid methyl ester content analysis. The experiment was terminated on day 7.

### 2.3. Fatty acid methyl ester (FAME) content analysis

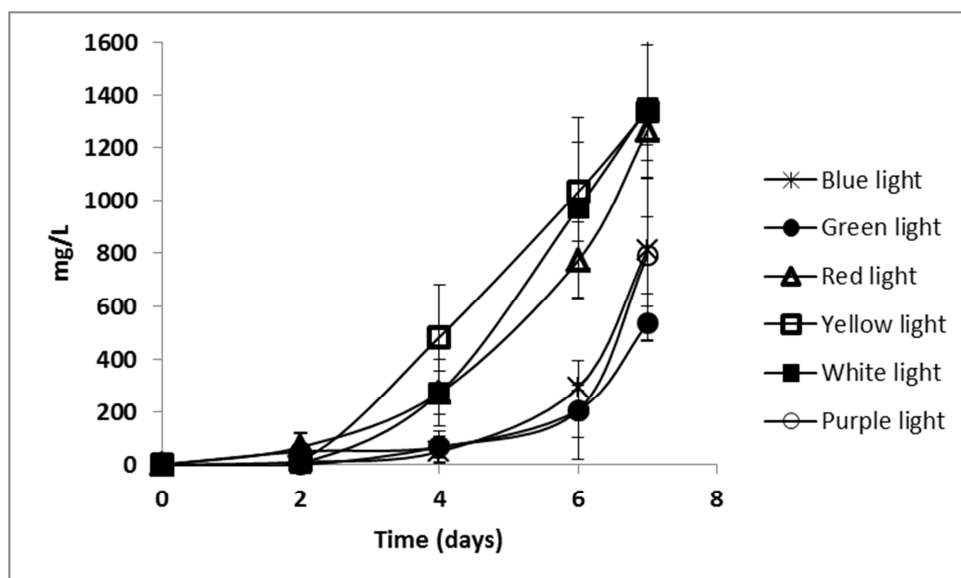
For each sample, 5 mg of the lyophilised algal biomass collected on day 7 was treated with 2 mL methanolic  $\text{H}_2\text{SO}_4$  (2% v/v) for 60 minutes at 90°C. The fatty acid methyl esters (FAME) were then extracted with hexane and analysed by GC as described by Thomæus et al. (2001). For quantification, heptadecanoic acid methyl ester was added as an internal standard.

### 2.4. Statistics

Each experiment was carried out with triplicate treatments, and mean and standard deviation are reported. The entire experiment was then repeated once. The data were analysed by analysis of variance followed by Tukey's multiple comparison test and differences were considered significant at  $P < 0.05$  (Minitab, version 16).

## Results and discussion

The growth of *C. vulgaris*, measured as dried biomass, was affected by light quality and the treatments exposed to yellow, red and white light reached the log phase earlier than the other treatments (Fig. 1).



**Fig. 1** Growth of *Chlorella vulgaris* (mg DW L<sup>-1</sup>) exposed to different quality of light. Error bars are shown when the standard deviation exceeds symbol dimensions. (Figure from Hultberg et al., 2014a)

There was also a significantly higher amount of biomass in these treatments after 7 days of growth than in blue, green and purple light (Table 1). These results confirm findings from a recent study on cultivation of *C. vulgaris* in wastewater using LED light (Yan et al., 2013). Red light is commonly suggested as optimal for biomass productivity in *C. vulgaris*, since this microalga has a high concentration of chlorophyll and hence absorbs efficiently in the red wavelength area (Fu et al., 2012).

**Table 1** Amount of biomass (mg DW L<sup>-1</sup>) and fatty acids ( $\mu\text{g mg}^{-1}$ ) produced by *Chlorella vulgaris* after seven days of growth under different quality of light

Treatment	Biomass <sup>i</sup> (mg DW L <sup>-1</sup> )	Fatty acids <sup>i</sup> ( $\mu\text{g mg}^{-1}$ )
White light	1337.5±250.9ab	67.9±6.0a
Blue light	813.3±338.6bc	63.8±7.3ab
Green light	633.3±64.3c	54.4±4.7b
Yellow light	1596.7±260.8a	67.7±5.6a
Red light	1263.3±55.1ab	64.8±8.3ab
Purple light	793.3±146.4bc	71.2±2.7a

<sup>i</sup>DW = dry weight. Values within columns followed by different letters are significantly different ( $P < 0.05$ , Tukey's test). (Table from Hultberg et al., 2014a)

Also significant differences in total lipid content (measured as sum of FAMES) were observed between treatments. The lipid concentration in *C. vulgaris* cultivated under green light was significantly lower (5% of cellular dry weight (CDW)) than that observed in the white, yellow or purple light treatments (7% of CDW) (Table 1). In a previous study using a mixed culture of *Chlorella* sp. and *Saccharomyces cerevisiae*, Shu et al. (2012) concluded that blue light is more favourable for accumulation of lipids than red light. That finding was not supported in the present study using a monoculture of *C. vulgaris*, where there

were no significant differences between treatments with red or blue light, both of which resulted in a total lipid content of approximately 6% of CDW. The lipid concentration was generally low in the present study, but the results suggest that light quality can be used together with other measures, such as strain selection and nutrient depletion, to increase total lipid content.

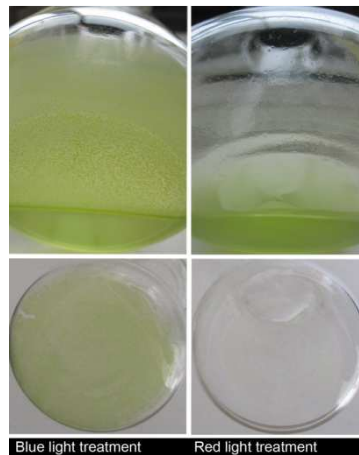
The fatty acid profile of *C. vulgaris* was also affected by light quality as presented in table 2. This effect was most obvious in the treatment with green light, where the concentration of hexadecatrienoic acid (16:3) was significantly increased and the concentrations of stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) were significantly decreased compared with in the other treatments. In addition, the highest concentration of  $\alpha$ -linolenic acid (18:3) was found in the algal biomass grown under green light. Both hexadecatrienoic acid (16:3) and  $\alpha$ -linolenic acid (18:3) are present in high concentrations in the thylakoid membranes of the chloroplasts in plants and also in green algae such as *C. vulgaris* (Hugly and Somerville, 1992; Nichols, 1965). The increased proportion of these fatty acids in the green light treatment could have been due to a compensatory increase in the amount of chloroplasts and/or a re-arrangement of the thylakoid structure within the chloroplast as a response to the low absorbance of this wavelength. Green light induces shade responses in plants and one of these responses is that the grana stacks in shaded leaves contain more thylakoid membranes than those in sun-exposed leaves (Zhang et al., 2011).

**Table 2** Relative proportions of the major fatty acids (% of total fatty acids) in *Chlorella vulgaris* after seven days of growth under different quality of light

Fatty acid	White	Blue	Green	Yellow	Red	Purple
16:0	16.7a	18.4ab	17.2ab	20.4b	19.6ab	18.1ab
16:1	3.4a	3.5a	4.1a	3.4a	2.2a	3.7a
16:2	1.8a	1.5a	2.1ab	2.6c	2.2b	1.8a
16:3	17.6a	17.7a	20.2b	16.5a	16.0a	16.6a
18:0	5.2a	6.5a	2.4b	4.9a	5.6a	6.5a
18:1	2.6a	3.5b	2.0c	2.4a	2.8a	2.8a
18:2	7.2a	6.8a	4.2b	7.7a	7.0a	7.4a
18:3	44.4ab	44.3ab	47.0a	40.7b	42.3b	42.0b

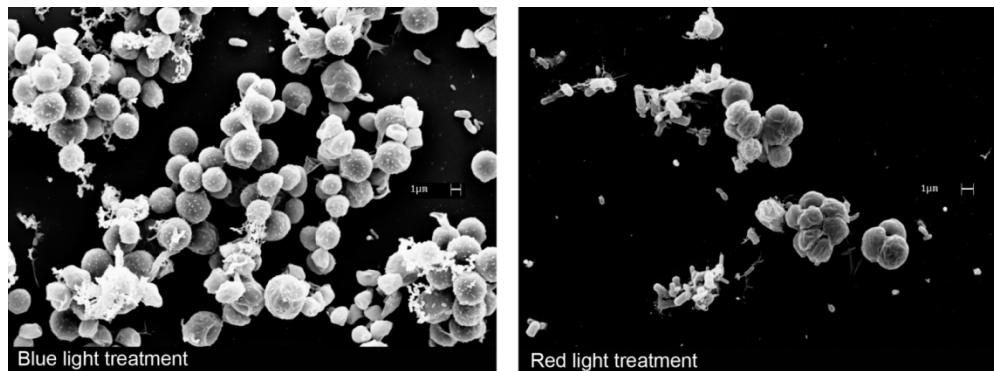
Values within rows followed by different letters are significantly different ( $P < 0.05$ , Tukey's test).  
(Table from Hultberg et al., 2014a)

For the treatments which were not aerated a clear visual difference in biofilm formation between the different treatments was observed by the end of the experiment. In the treatments exposed to red, yellow and green light, it was evident that less biofilm had formed after 7 days of algal growth compared with in the treatments exposed to blue, purple and white light (Fig. 2).



**Fig. 2** Biofilm formation by *Chlorella vulgaris* after 7 days of algal growth in the treatments with blue and red light. In terms of visual appearance, the treatments where blue, purple and white light were used resulted in high biofilm formation, whereas the treatments with red, yellow and green light resulted in less biofilm formation. (Figure from Hultberg et al., 2014b)

When glass surfaces from the different treatments were examined by light microscopy, the treatments exposed to blue, purple or white light showed the presence of biofilm formation already after 4 days of growth. This was not observed for the other treatments. Light microscopy analysis of the cells in the medium showed that the cells grown in blue, purple or white light were more often aggregated into large groups, whereas the cells grown in red, yellow and green light were mostly present in a solitary state. This difference between the treatments was evident already after 4 days of algal growth. Scanning electron microscopy of the glass surfaces after 7 days of growth confirmed these results (Fig. 3).



**Fig. 3** Scanning electron micrographs of the biofilm formed by *Chlorella vulgaris* after 7 days of algal growth in the treatments with blue and red light. There was dense colonization of the glass surface by microalgae when blue light was used for cultivation and only occasional grouping when red light was used. Scale bar 1 µm. (Figure from Hultberg et al., 2014b)

An interesting application of these findings would be manipulation of light quality for harvest of microalgae. A bottleneck in the development of new algal culturing systems is the harvest methods existing today, filtering, chemical flocculation and centrifugation, which are both expensive and energy-demanding (Uduman et al., 2010). Light could be used for stimulating growth as a biofilm for easy harvest, or to increase aggregation of the cells so they can be removed by filtration. In this regard, it is of interest to note that the total amount of biomass was significantly



higher in the treatments where biofilm formation was observed. It is unclear whether biofilm formation is a density-dependent process or there is increased growth under certain light quality. However, the fact that aggregation of the cells and biofilm formation was seen already after 4 days of growth under a certain light quality indicates that this is the more important factor.

High biofilm formation was observed in the treatments receiving either blue light only or white and purple light, which contain parts of blue light. This suggests that it was the blue light that stimulated biofilm formation. From studies on prokaryotes it is known that different classes of blue-light receptors can be involved in biofilm formation (Gomelsky and Hoff, 2011). These studies have shown that the response is not uniform among the prokaryotes, with both blue-light activated biofilm formation (Purcell et al., 2007) and blue-light inhibition of biofilm formation (Mussi et al., 2010) having been observed. Our study was performed using a freshwater green algal species and further work is needed using different microalgal strains to determine whether blue light stimulation of biofilm formation is a general phenomenon.

## Conclusion

This study showed that light quality significantly affected biomass productivity in the green alga *C. vulgaris* and also total lipid concentration and fatty acid profile. At present, these results cannot be directly transferred to other microalgal species. However, they demonstrate the potential for including LED technology among other measures when designing customised cultivation systems for optimal microalgal production, whether targeting biomass productivity or selected metabolites. The results also clearly imply that light quality plays a role in the formation of microalgal biofilms. In practice, this process is of high importance and there are major practical implications of these results. The potential for using light quality as a tool for increasing or decreasing biofilm formation needs to be further studied.

## Publications in the project

Two scientific papers based on this project have been published in 2014. Ångpanneföreningen's Foundation for Research and Development, Sweden, is acknowledged in both papers.

- Hultberg M, Asp H, Marttila S, Bergstrand KJ, Gustafsson S (2014) Biofilm formation by *Chlorella vulgaris* is affected by light quality. *In press* Current Microbiology, DOI 10.1007/s00284-014-0645-1
- Hultberg M, Larsson Jönsson H, Bergstrand KJ, Carlsson AS (2014) Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*. *Bioresource Technology* 159: 465-467

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## Financial report

A total of 325 000 SEK was supplied for this study. This funding has been used as described below.

<b>Costs</b>	<b>SEK</b>
Salary (M. Hultberg)	160 000
Materials (Algae, Chemicals, Laboratory equipment)	20 000
Analysis	28 000
Rent (Office and growing chambers)	38 000 (29 + 9)
Publication costs	11 000
Overhead	68 000
<b>Total costs</b>	<b>325 000</b>