HZ University Of Applied Sciences

Robust algae for aquaculture

Research to qualitative and quantitative cultivation of marine algae Rhodomonas sp.

G.P. Verbeeke
4th of June 2017

ROBUST ALGAE FOR AQUACULTURE

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Bachelor thesis

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HZ University of applied sciences

Delta academy

Research group Aquaculture

Aquatic Ecotechnology

Version 4

4th of June, 2017

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Preface

In front of you lays the thesis 'Robust algae for aquaculture'. I wrote this thesis as a part of my bachelor study Aquatic Ecotechnology at the HZ University of applies sciences and in collaborati on with the research group Aquaculture. I have been actively working on this thesis in the period of February 2017 until mid-June 2017.

The research question for this thesis was formulated as *'What is the effect of light, both wavelength and intensity, on the growth rate and quality of the cultivated marine microalgae Rhodomonas sp. for the application in the aquaculture?'.* In order to answer this question, numerous experiments and analyses needed to be performed, which eventually yielded an answer to this research question. However, this research would not have been able without the help of certain people.

Therefore, I would like to thank my mentor and examiner, Alco Nijssen, at the HZ university of applied sciences for his advice and help. Furthermore, I would like to thank the Aquaculture research group for the possibility to carry out this research. Very special thanks goes to my supervisor, Christos Latsos, for his immense amount of patience, his guidance, the possibility to always ask questions and the fun times in the Sea lab. He was able to bring out the best in me and took me and this research to a higher level.

In addition I would like to thank my fellow students in and outside the office for all the good times, the laughs, the discussions and shared stress moments. Especially I want to thank Bo Schreur for being such a good friend and keeping an eye on me. Her numerous post-it's full with quotes, 'you can do its', balloons and hugs helped me through this final thesis! In addition, I want to thank A lya Alnabhani for all our 'serious' talks and discussions, and our dry humour in and outside the office. I will definitely miss these moments.

And last but not least, I want to thank my family for always supporting me and for the warm welcomes when I came home in the weekends.

Summary

The aquaculture sector is expanding worldwide, which gives this sector an important economic potential. A problem for the on land aquaculture sector is that there is insufficient quality feed for cultivating fish larvae and shellfish. Based on this problem the RAAQUA project was launched (Robust Algae cultivation for the Aquaculture) to realize a commercial achievable, qualitative high standard and stable algae cultivation which will improve the on land aquaculture production. The marine microalgae *Rhodomonas sp.* provides many benefits to the aquaculture, as feed but also in other applications. Therefore, it is necessary to design an applicable cultivation plan for this algae.

Rhodomonas sp. is a cryptophyte, a unicellular algae, that has specific characteristics. This algae consists of two plastids which contains the phycobilin protein phycoerythrin besides the common chlorophyll pigment. This additional protein acts as an antenna for harvesting light energy. Phycoerythrin is active at specific wavelengths in the visible light spectrum ranging from 460 to 610nm. Within this range phycoerythrin has three absorption peaks that can be found in the blue, green and yellow region of the spectrum. The position of this pigment in the chloroplast makes this algae special. Where pigments are normally positioned in the stromal side of the thylakoid, phycoerythrin is located at the lumen side. Only cryptophyte species have organized their pigmentation this way. The possession of the phycoerythrin protein makes it possible for *Rhodomonas sp.* to live in the deeper regions of the ocean, which is unreachable by red light that is captured by the chlorophyll in green algae (which consequently live in the upper layers of the ocean). Blue light is made up of longer wavelengths that consist of more energy (40% more) than red wavelengths and is therefore able to reach these depths.

Several studies have been performed with respect to irradiance for *Rhodomonas sp.* However, as of yet no study to the optimal cultivation wavelength has been performed for cryptophytic species, although wavelength studies have been done on green algae blue green algae and cyanobacteria. Therefore, the aim of this present study was to investigate what light intensity in combination with wavelengths are the most suitable for cultivating *Rhodomonas sp.* with respect to its growth and biochemical composition. Importantly, the findings of this study will help designing a quantitative and qualitative high and stable cultivation plan for *Rhodomonas sp. Rhodomonas sp*. was cultivated under three light conditions, low light (8 ± 10 µmol m^{-2} s^{-1}), medium light (60 ± 10 µmol m^{-2} s^{-1}) and high light (80 ± 20 µmol m^{-2} s^{-1}) while being exposed to wavelengths representing the colours red (λ_{peak} 630 nm), green (λ_{peak} 517 nm), blue (λ_{peak} 461 nm) and white as reference (λ range of 461 – 630 nm). *Rhodomonas sp.* was batch cultured (150ml) under continuous irradiance at 20 °C (salinity of 30 g L^{-1}) in an incubator. Several parameters were monitored including biomass, productivity, cell density and the biochemical composition. The biochemical composition monitoring consisted of chlorophyll a and b, carotenoids, phycoerythrin, carbohydrate and protein content analysis.

This study has demonstrated that cultivating *Rhodomonas sp.* with an irradiance of 60 µmol m^2 s^{-1} under blue wavelengths of light results in the highest biomass (1.64 g L^{-1}), maximum productivity (0.58 g L^{-1} day^{-1}) and chlorophyll a content (1.1 pg $cell^{-1}$). In addition, the highest chlorophyll b (10.2 pg $cell^{-1}$), carbohydrate (88 pg $cell^{-1}$) and protein (496 pg $cell^{-1}$) levels are found under the same light intensity for the colours red, green and white respectively. Phycoerythrin and carotenoids content will be the highest when *Rhodomonas sp.* is cultivated with a PFD of 8 µmol m^2 s^{-1} with blue (19.6 pg $cell^{-1}$) and red (8.6 p $cell^{-1}$) light respectively.

In elaboration of this present study there is recommended to do more research to protein and carbohydrate behaviour in respect to growth phases under different light conditions for *Rhodomonas sp.* In addition, the effect of combining colours, changing colours per growth phase and colours in respect to the absorption range of the photosynthetic pigments on the cultivation of *Rhodomonas sp.* is recommended to investigate further. Moreover, implementing a light cycle might induce pigmentation and growth. At last, up scaling of the cultivation with the conditions found in the present study in continuous systems or photo bioreactors is recommended.

Samenvatting

De aquacultuur sector wereldwijd aan het uitbreiden. Dit geeft deze sector een belangrijk economisch potentieel. Een probleem voor binnendijkse aquacultuur is dat er niet tot nauwelijks genoeg voedsel is voor het opkweken van vis larven en schaaldieren. Mede dankzij deze problemen is het RAAQUA project opgesteld (Robuuste algen kweek voor in de aquacultuur). Het doel van dit project is om een commercieel haalbaar cultiveerplan te ontwikkelen met hoge standaarden voor zowel kwaliteit als kwantiteit. De uitkomsten van dit project zullen de productie in de binnendijkse aquacultuur een boost geven. De marine micro alg *Rhodomonas sp.* heeft vele voordelen binnen de aquacultuur, zowel als voedsel als in andere applicaties. Het is daarom ook van essentieel belang dat er voor deze alg een toepasbaar cultiveerplan wordt ontwikkeld.

Rhodomonas sp. is een cryptophyte, een eencellige alg die specifieke karakteristieken heeft. Deze alg heeft twee plastiden welke naast de normaal voorkomende chlorofyl pigmenten ook een phycobilin eiwit hebben, namelijk phycoerythrin. Dit pigment dient als een opvangnet voor licht energie. Phycoerythrin is actief in het zichtbare licht spectrum tussen een golflengte van 460 tot 610 nm. In dit gebied heeft phycoerythrin drie absorptie pieken die zich in het blauwe, groene en gele gebied bevinden. De positie van deze pigmenten in de chloroplast maakt deze algensoort bijzonder. Waar de pigmenten zich normaal in het stroma bevinden, zijn de phycoerythrin pigmenten te vinden in de lumen zijde van de thylakoïde. Wat deze algensoort speciaal maakt is dat alleen in cryptophyte soorten de pigmenten op deze wijze geordend zijn. *Rhodomonas sp.* leeft in de diepere gebieden van de oceaan, waar phycoerythrin het mogelijk maakt om te overleven. Rood licht dat wordt opgenomen door chlorofyl in groenalgen (oppervlakte organismen) wordt of opgenomen of dringt niet door tot deze diepere lagen van de fotische zone. Blauw licht, welke een hogere concentratie aan energie heeft (40% meer dan in rood licht) en een langere golflengte, dringt wel diep door en bereikt de diepte waar de *Rhodomonas sp.* zich bevindt.

Verschillende onderzoeken zijn uitgevoerd met betrekking tot de lichtsterkte voor de *Rhodomonas sp.* Echter, wat de meest optimale golflengte voor het kweken van deze alg is, is nog niet bekend. Zulk onderzoek is wel gedaan naar groen alg, blauwalg en cyanobacteriën. Daarom is er in dit huidige onderzoek onderzocht welke licht sterkte en golflengte het meest optimaal zijn voor het kweken van *Rhodomonas sp.* met betrekking tot zijn groei en biochemische samenstelling. De resultaten van deze studie zullen bijdragen aan het creëren van een kwantitatief, kwalitatief en stabiel kweekplan voor deze alg. *Rhodomonas sp.* is gekweekt onder drie lichtsterktes, lage licht intensiteit (8 ± 10 µmol m^{-2} s^{-1}), middelmatige licht intensiteit (60 ± 10 µmol m^{-2} s^{-1}) en hoge licht intensiteit (80 ± 20 µmol m^{-2} s^{-1}) met golflengtes welke de kleuren rood (λ_{piek} 630 nm), groen (λ_{piek} 517 nm), blauw (λ_{piek} 461 nm) en wit als referentie (λ gebied van 461 – 630 nm) representeren. *Rhodomonas sp.* werd in batches (150 ml) gekweekt in een incubator onder constante belichting met een temperatuur van 20 °C en een saliniteit van 30 g L^{-1}. Er werd toezicht gehouden op verschillende parameters waaronder biomassa, productiviteit, cel dichtheid en de biochemische samenstelling. Voor de biochemische samenstelling werd er gekeken naar de chlorofyl a en b, caroteen, phycoerythrin, koolhydraten en eiwit gehalte.

Dit onderzoek laat zien dat het kweken van *Rhodomonas sp.* onder blauw licht met een licht sterkte van 60 µmol m^2 s^{-1} de hoogste biomassa (1.64 g L^{-1}), productiviteit (0.58 g L^{-1} day^{-1}) en chlorofyl a (1.1 pg cel^{-1}) gehalte geeft. Met dezelfde lichtsterkte wordt het meeste chlorofyl b (10.2 pg cel^{-1}), koolhydraten (88 pg cel^{-1}) en eiwit (496 pg cel^{-1}) gehalte gevonden met een blootstelling aan respectievelijk rood,

groen en wit licht. Het phycoerythrin en caroteen gehalte is het hoogst met een lichtsterkte van 8 µmol m^2 s^{-1} wanneer de alg wordt blootgesteld aan respectievelijk blauw (19.6 pg cel^{-1}) en rood (8.6 pg cel^{-1}) licht.

De aanbevelingen na de verkregen resultaten zijn om verder onderzoek te doen naar het gedrag van eiwitten en koolhydraten in respect tot de groei fases wanneer *Rhodomonas sp.* gecultiveerd word onder verschillende licht condities. Verder wordt het aangeraden om onderzoek te doen naar het combineren van kleuren, het aanpassen van kleuren per groei fase en om te kijken naar de kleuren in de absorptie pieken van de fotosynthetische pigmenten. Ook het gebruik maken van een licht cyclus voor het cultiveren van *Rhodomonas sp.* kan interessant zijn. Als laatste wordt aanbevolen om met de gevonden licht condities de cultivatie op te schalen in continue systemen of fotobioreactoren.

Table of content

List of figures

List of tables

List of abbreviations

Chl a :	Chlorophyll a
Chl b:	Chlorophyll b
HL:	High Light
LED:	Light Emitting Diode
LHC:	Light harvesting complex
LL:	Low Light
ML:	Medium Light
PBS:	Phycobilisome
PE:	Phycoerythrin
PFD:	Photon Flux Density
RAAQUA:	Robust Algae for Aquaculture
RC1:	Reaction centre 1
RC2:	Reaction centre 2
WGRB LED:	White Red Green Blue Light Emitting Diode

1. Introduction

The aquaculture sector is expanding worldwide, which gives this sector an important economic potential (Merino et. al., 2010). Marine and fresh water species are cultivated within the aquaculture sector. Important products are fish larvae and shellfish which are sold on national and international level.

The aquaculture sector can be divided in two categories, production on land and on the sea side. On land production is the cultivation of plants or organisms on the dry side of the dike in greenhouses, buildings or on the land. Benefits of on land cultivation are that the quality and the amount of the production can be controlled. The drawback is that there is not enough or no feed for the cultivation of fish larvae and shellfish (Rijksoverheid, 2015). Therefore, it is important that the aquaculture companies have access to feed the whole year round. For fish, larvae and shellfish this means that there must be enough microalgae available. Nevertheless, not only the availability is important, the quality of these microalgae is key for the growth ratio of the fish larvae and shellfish. The higher the growth ratio, the better the production (Seixas et. al., 2009). An alga species that is often used in the Aquaculture sector as feed is the *Rhodomonas sp.* With the current systems for algae production it is not possible yet to realise a stable algae cultivation with high quality against an acceptable cost price for this species. Based on this the RAAQUA project was launched (Robust Algae cultivation for the Aquaculture). The goal of this project is to investigate and realize a commercial achievable, qualitative high standard and stable algae cultivation which will improve the on land aquaculture production. Within the RAAQUA project different control variables are investigated (light, temperature, medium, harvest regime, mixing) and determined the effects that these variables have on the quantity, cost price and quality of microalgae cultivation. The quality of the microalgae depends on the amount of energy and nutrients that fish larvae and shellfish can metabolize out of it. In the investigation from Patil et al. (2006) it appears that *Rhodomonas sp.* (Cryptophyceae, figure 1) has a diverse content of fatty acids and therefore is very suitable for fish larvae and shellfish cultivation. An important feature in algae species is the content of light harvesting pigments. *Rhodomonas sp.* contains, besides the common pigments as chlorophyll, a phycobilin, named phycoerythrin, that harvest light at a specific wavelength (Doust et. al., 2006). Phycoerythrin is highly valuable (30–150$/mg) for different applications, such as fluorescent agent (Chaloub et al., 2015) or in food applications (Sudhakar et al., 2015).

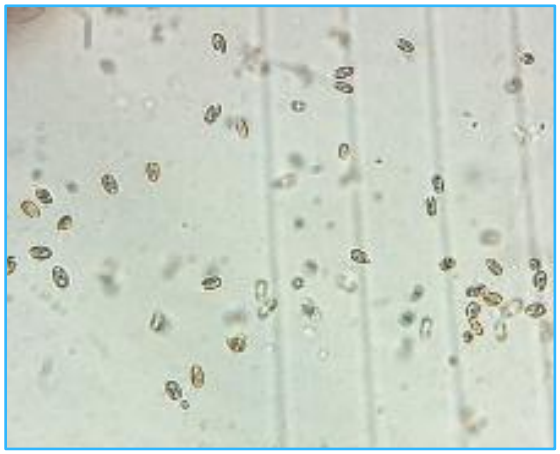

***Figure 1**: Rhodomonas sp. photographed trough a light microscope, 100x magnification.*

Because microalgae are photoautotroph (they are dependant of (sun)light), light is one of the control variables that is being investigated within the RAAQUA project.

1.1 Problem formulation

One advantage of cultivating *Rhodomonas sp.* is their ability to sustainably produce proteins, carotenoids, fatty acids and carbohydrates. This makes this algae very suitable as feed in the aquaculture sector (Seixas et al., 2009), but also for other applications in biomedical, biotechnological and nutraceutical sectors (Manirafasha et.al., 2016). However, there is currently no commercially stable cultivation plan that can be applied within the aquaculture sector in order to produce this algae with a high quality for an acceptable cost price. Cultivation conditions in respect to light wavelengths has been investigated for green algae (Baba et. al., 2012; Mohsenpour et. al., 2012; Yan et. al., 2013; de Mooij et. al., 2016), blue green algae (Madhyastha et. al., 2009) and cyanobacteria (Mohsenpour et. al., 2012). However, despite the absorption characteristics of phycoerythrin, literature is not found for cryptophytic species in respect to cultivation under different light wavelengths. Therefore, to design a cultivation plan for *Rhodomonas sp.* it is important to investigate the effect of different light conditions.

1.2 Goal

The goal of this research was to determine the light conditions for optimizing the quantity and quality of *Rhodomonas sp.* There has been investigated which light wavelength and intensity is optimal for the growth of the algae *Rhodomonas sp.* Growth is defined by productivity, cell density and dry weight. In addition, there was investigated how this affects the quality of the algae, defined by analysing the biochemical composition (light harvesting pigments, carbohydrates and proteins).

1.3 Research question

The next research question was formulated:

'What is the effect of light, both wavelength and intensity, on growth and quality of the cultivated marine microalgae Rhodomonas sp. for the application in the aquaculture?'.

This research question was divided in three sub questions:

- Which wavelengths of light (representing the colours red, blue, green, or white) will give the best growth of the marine algae *Rhodomonas sp.*?
- Which light intensity is the most suitable for the cultivation of *Rhodomonas sp.* in respect to growth?
- What will the quality of *Rhodomonas sp.* be with the chosen light wavelength and intensity defined by concentrations of light harvesting pigments, proteins and carbohydrates?

This research will show which light wavelength and intensity is optimal for the growth of the algae *Rhodomonas sp.* and how this effect their quality defined by concentration of light harvesting pigments, carbohydrates and proteins.

2. Theoretical framework

Table 1: Taxonomic data of the marine algae Rhodomonas baltica

Empire	Eukaryota
Kingdom	Chromista
Phylum	Cryptophyta
Class	Cryptophyceae
Order	Pyrenomonadales
Family	Pyrenomonadaceae
Genus	*Rhodomonas*

The marine alga *Rhodomonas sp.* (Karsten 1898) is a cryptophyte, for full taxonomic data see table 1. Cryptophytes have specific characteristics, they are unicellular algae and move through the water with the use of two unequal flagella (dinoflagellate). Another characteristic is that the algae cells do not have a cell wall. Instead, they are covered by a periplast, which forms a complex surface structure in combination with the cell membrane (Hausmann, 1979). *Rhodomonas sp.* size ranges from 7 up to 14 µm and thrives the best at a temperature between 19 - 26°C. Each cell contains one plastid (in some cases two) where the pigments are located. In addition of the common chlorophyll and carotenoid pigments (chlorophyll a and c; β-, α-carotene), the pigment phycoerythrin is present in *Rhodomonas sp.* plastids (Doust et al., 2006). The *Rhodomonas baltica* has a high content of polyunsaturated fatty acids: DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) (Patil et al., 2006). These characteristics make *Rhodomonas sp*. valuable as feed for copepods, artemia nauplii, shellfish, zooplankton and other filter feeders.

There are three major classes of photosynthetic pigments that occur among algae: chlorophylls, carotenoids and phycobilins (Rowan, 1989). Phycoerythrin is a phycobilin, a complex (red) photoreceptor pigment, that together with phycocyanin (blue pigment) and allophycocyanin (bluish, green) forms water soluble proteins called phycobiliproteins (Glazer & Stryer, 1984). In addition, these proteins can aggregate to a highly ordered protein complex called a phycobilisome, which makes phycobilins unique among photosynthetic pigments (figure 2). Each phycobilin has its own absorption maxima which range between 540 – 570 (colour: green – amber), 610 – 620 (colour: Amber – orange) and 650 – 655 nm (colour: red) for phycoerythrin, phycocyanin and allophycocyanin respectively (Viskari & Colyer, 2003).

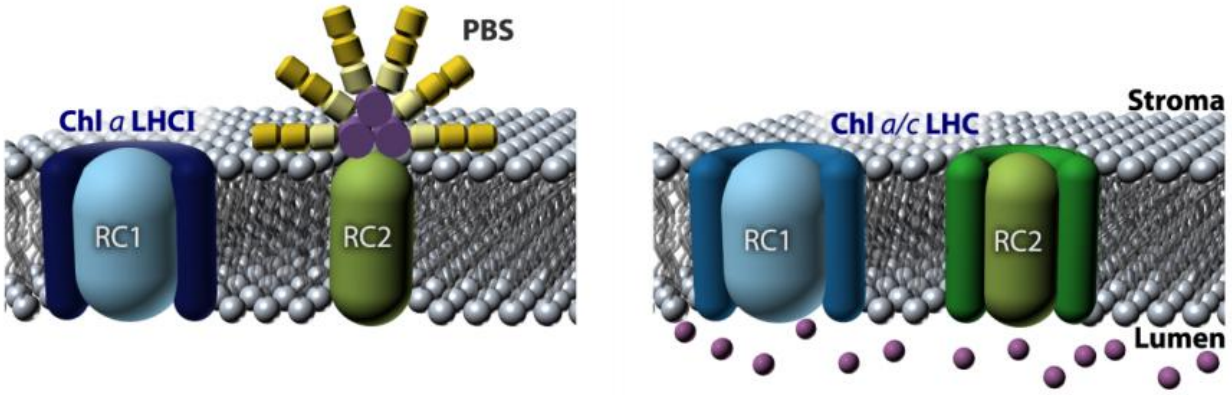

Figure 2: *Structure of the light harvesting complexes (LHC) in the thylakoid of the chloroplast of a Rhodophyte (left) versus a Cryptophyte (right). The reaction centres are shown in blue and green, RC1 and RC2 respectively. On the Rhodophyte LHC a phycobilisome (PBS) is attached to RC2. This phycobilisome consist of phycoerythrin (yellow), phycocyanin (light yellow) and allophycocyanin (purple). In the Cryptophyte algae only the phycobilin phycoerythrin can be found (purple), where it is located in the lumen of the thylakoid. Source: Mirkovic, 2009.*

These phycobilisomes are located in the chloroplast (figure 3) where they are attached to the stromal face of the thylakoid. The phycobilins, aggregated as phycobilisomes or not, serve as an antennae, it captures the light energy and transfers it to the thylakoid which is the main structure for photosynthesis. The light harvesting antennas are very important for photosynthetic organisms. The reaction centre would not have a steady supply of excitation without these antennas since the light harvesting pigment chlorophyll is inefficient when looked at the absorbance abilities. Chlorophyll a is not able to absorb light at the peak solar output, in addition a chlorophyll molecule is limited in absorbing photons, it is only able to absorb a few photons at the time (Mirkovic, 2009). The light harvesting antennas captures the photons with the correct energy and transfers them to the reaction centre. The energy travels as follows: phycoerythrin → phycocyanin → allophycocyanin → photosynthetic reaction centre (Glazer & Stryer, 1984). The photosynthetic reaction centre is a complex system where primary energy conversion reactions of photosynthesis take place (Mirkovic, 2009). It consists of two reaction centres, reaction centre 1 and 2, where photochemical reactions traps the energy of the photon (Heathcote et. al., 2002).

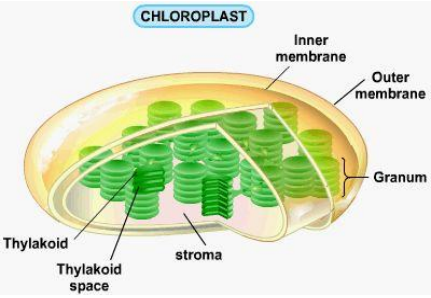

Figure 3: *Cross section of a chloroplast. The phycobilisomes are located on the stromal side of the thylakoid and transfer the captured light energy to the antennae.*

One of the characteristics that makes a cryptophyte differ from other algae, is the composition of the light harvesting complexes. In all cryptophytes, in contrast to other algae groups, the phycobilins are located in the lumen, also called thylakoid space, of the chloroplast (figure 2, 3) (Mirkovic, 2009). Cryptophyte species can have phycoerythrin or phycocyanin as a light harvesting antenna, but never both at the same time. The cryptophyte *Rhodomonas sp.* contains phycoerythrin as a light harvesting antenna. Phycoerythrin has a absorption maximum at a wavelength of 545nm where it absorbs in the green spectral area, see figure 4 (van der Weij-De Wit et al., 2006).

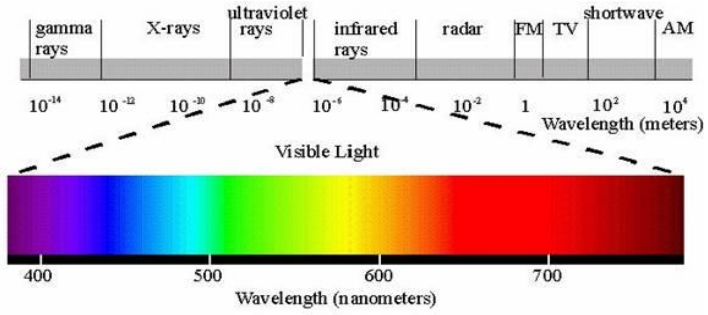

Figure 4: *Overview of the electromagnetic spectrum, with the visible spectrum highlighted.*

The pigment phycoerythrin enables algae to be efficient in deep water of the photic zone (Doust et al., 2006). Light energy that is needed for chlorophyll, red light, which is at the end of the visible spectrum and has longer wavelengths and therefore contains less energy, is absorbed by the green algae in the first layers the photic zone. The light that penetrates deeper, high energy containing short wavelengths of blue/green light, will be absorbed by phycoerythrin which harvest light at a absorption where chlorophyll is inefficient (Mirkovic, 2009). In the study of Viskary & Colyer (2003) the absorption rates of the different major photosynthetic pigments are compared. It shows that where chlorophyll and carotenoids have a low absorbance efficiency, the phycobilins have a high absorbance, figure 5.

According to this, the wavelength of light is important for an efficient transfer of energy to the photosynthetic reaction centre where this energy will be converted to biomass. However, the intensity and density of light are also important factors (Chaloub et al., 2015). The photosynthetic photon flux density (PFD), the density of photons in the photosynthetic active light spectrum, can affect the biomass production and fatty acid production of algae. A PFD of 600 µmol m^{-2} s^{-1} gives an algae biomass production of 3,51 g m^{-2} d^{-1} versus 1.06 g m^{-2} d^{-1} with a PFD of 50 µmol m^{-2} s^{-1} (Biofilm of green algae) (Schnurr et al., 2016). In the same research is showed that light intensity has similar influence on the production of fatty acids. In addition, the duration of algae illumination is another factor which should be considered when cultivating algae (Schnurr et al., 2016). There are two ways to cultivate algae in respect to light intensity. One way is to continuously expose the algae to a light source, while another technique is implementing a dark/light cycle where there is x hours darkness and x hours light. The research of Chaloub et al. (2015) reveals that when algae are continuously exposed to a light source the cell density will be significant lower than when a dark/light cycle is applied. In addition, it shows that the amount of phycoerythrin is higher when using the light cycle (Chaloub et al., 2015).

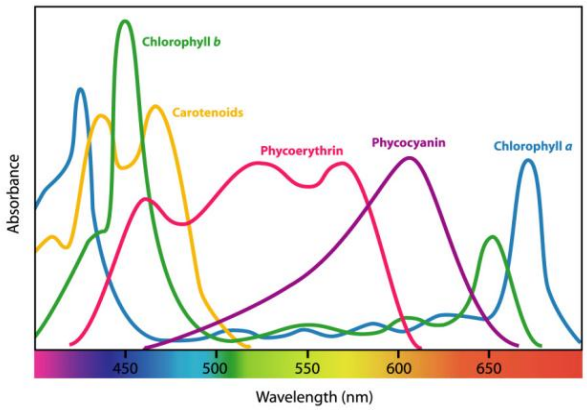

Figure 5: *The absorbance of the major photosynthetic pigments chlorophyll a - b (blue and green respectively), Carotenoids (yellow), the phycobilins phycoerythrin (pink) and phycocyanin (purple).*

To cope with all these different factors, specific light sources are needed. There is a large amount of lamps commercially available with each different characteristics. Energy is needed to light these light sources, especially when continuously light exposure takes place. In the research of Blanken et al. (2013) is investigated what the energy balance is between the input of energy for the light source against the uptake of energy by the photosynthetic reaction centre of the algae. Only 4 – 6% of electrical energy input will be conserved in the microalgae (Blanken et. al., 2013). One of the light sources used for cultivating algae are light emitting diodes (LED's). LED's are able to emit a narrow band of wavelength, which makes them an efficient light source for cultivating algae in relation to the specific absorption spectra of the light harvesting pigments (Yan et al., 2013). In addition, LED lights can be processed in almost all cultivation systems and are low in energy consumption. Ordinary lights, for example light bulbs or fluorescent lamps, have a broader spectra which inefficiently covers the absorption spectra of the pigments and are therefore not suitable for cultivating algae (Wishkerman & Wishkerman, 2017).

Different cultivation techniques are available for growing microalgae. A division is often made between open and (semi)closed cultivation systems, where open systems can be raceways or ponds and closed systems different forms of photo-bioreactors. The open cultivation systems mainly focus on large scale microalgae cultivation, however, the parameters are often difficult to control and the cultures are exposed to external contamination (Borowitska, 2005). In photo-bioreactors the cultivation parameters can be highly controlled, although the disadvantage is that in most cases artificial light is needed to illuminate the cultures (Behrens, 2005).

Algal cultures grow according to certain phases which forms a characteristic growth curve. There are four distinct phases consisting the lag phase, the exponential phase, the stationary phase and the end phase (see figure 6). In each phase the algae cells behave differently in respect to the metabolism and the biochemical composition (Lafarga-De la Cruz et al., 2006). During the exponential phase pigments are synthesised to obtain as much light energy as possible in order to convert the energy to biomass in combination with nutrients and other building blocks. However, when algae are batched cultured, nutrients can become a limiting factor and can influence the metabolism of the algae. In previous studies it has become clear that cryptophyte algae can store their pigments as nitrogen reserve when nitrogen limitation occurs (da Silva et. al., 2009).

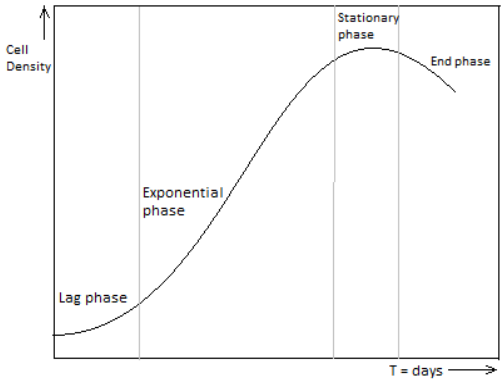

Figure 6: *Growth phases of an algal culture.*

2.1 Hypothesis

According to literature it is expected that *Rhodomonas sp.* would cultivate the best under blue (460 nm)/green (520 nm) light with a PFD of 100 – 200 m^2 s^{-1} because of the absorption range of the photosynthetic pigment phycoerythrin (active wavelength 440 – 570nm) that occurs in the algae. Thus because blue light has short wavelengths and contains more energy than the longer wavelength such as red (Whishkerman & Whishkerman, 2017). This characteristic makes the blue light very suitable for the phycoerythrin pigment. However, the expectations for the biochemical composition for the algae cells exposed to the different wavelength and intensities is more complex. Because the wavelengths of the blue light contains more energy than the red wavelengths of light, the expectation was that the cultures exposed to the red light needs more pigments than the cultures exposed to the blue light to capture the same amount of energy. In other words, there was expected that pigmentation in the cultures of red lights would be higher than in the cultures exposed to other light colours.

3. Materials and methods

3.1 Organism and stock culture conditions

The marine microalgae *Rhodomonas sp.* was obtained from the Roem van Yerseke B.V., Yerseke, Netherlands, and maintained in pre-sterilized 300 ml Erlenmeyer flasks (20 minutes at 120 °C) containing 150 ml of 6 times concentrated L1 medium with a salinity of 30 g L^{-1} (adapted from Guillard, Hargraves and Walne, 1993, appendix A). The medium was sterilized by filtering with a Sartorius liquid filter (0.2µm pore size) in the sterilized Erlenmeyer flasks. The cultures were maintained in an incubator at 20 ± 1 °C and CO_2 supply. Sodium bicarbonate (8 M) was added to the L1 medium to prevent pH differences during cultivation. All the stock cultures were continuous illuminated at an irradiance of 120 µmol m^2 s^{-1} provided by TL fluorescent tubes. The growth of the cultures were followed by measuring the cell density with a Coulter counter (Beckman coulter Z1). Once every week sub cultures were made from the stock cultures.

3.2 Experimental conditions

Three experiments were conducted in which *Rhodomonas sp.* cultures were exposed to different light intensities ranging from 8 ± 10, 60 ± 10 and 80 ± 20 µmol m^2 s^{-1} for experiment 1, 2 and 3 respectively. The light intensity was measured and monitored manually with the help of a light meter (Skye).

In each experiment, 1 – 3, the culture conditions were the same. *Rhodomonas sp.* was batch cultured in pre-sterilized 300 ml Erlenmeyer flasks (20 minutes at 120 °C) containing 150 ml of 6 times concentrated L1 medium (salinity 30 g L^{-1}) with 8 M sodium bicarbonate at 20 ± 1 °C in a CO_2 incubator. The experiments were started with a culture density of 400 ± 100 thousand cells ml^{-1} (10% of medium volume). All experiments were conducted in triplicates and the cultures (n = 3) were exposed to 4 different wavelengths emitted by WRGB LED lights representing the colors blue, green and red (λ_{peak}:461, 517 and 630nm respectively) with white LED light (λ_{range}: 415 – 720nm) as reference, figure 7. A structure was made to prevent light pollution by the different colors, figure 8.

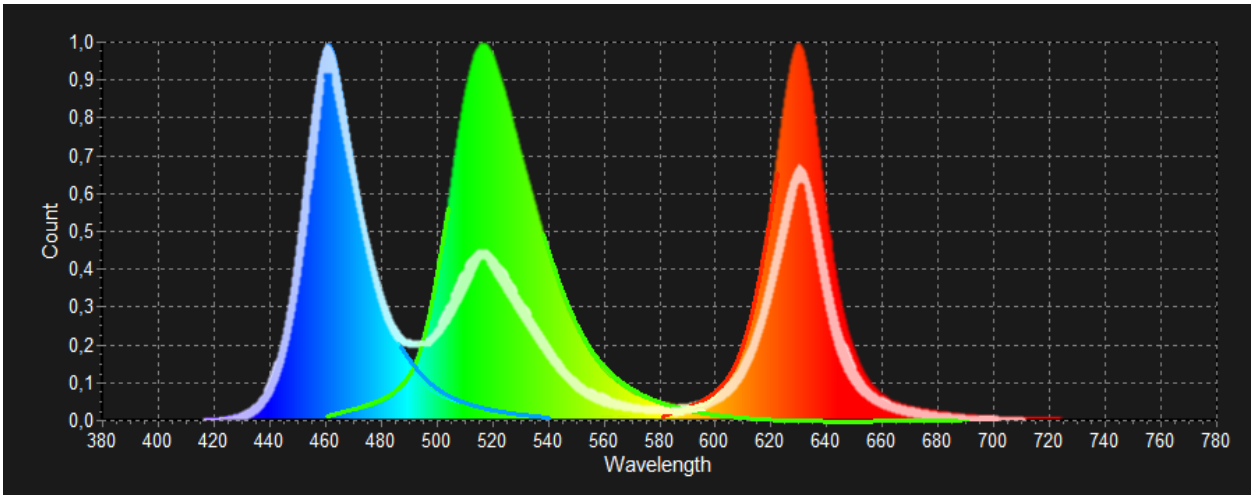

Figure 7: *Wavelength range for the WRGB LED lights. Corresponding colour lines represents the wavelength range.*

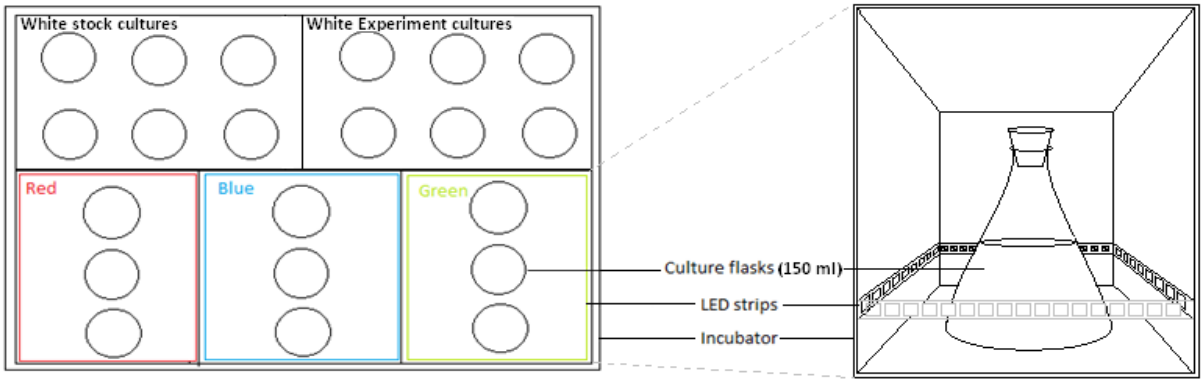

Figure 8: *Experimental setup for all experiments.*

3.3 Culture analysis

Each culture, in total 12 per experiment, was monitored by measuring daily the cell density, size 7 – 14 µm, in triplicate (coulter counter, Beckman Coulter Z1) and the optical density in duplicate (spectrophotometer, DR 5000).

Prior to this research the dry weight of different culture densities of *Rhodomonas sp.* was determined and plotted in a graph to retrieve the formula for converting the optical density to dry weight (appendix B). By measuring the optical density the dry weight could be monitored and the productivity and cell weight determined.

3.4 Biochemical analysis

In order to get knowledge of the quality of the cultures the biochemical composition was analyzed and different extractions were carried out. Pigments, consisting chlorophylls, carotenoids and phycobilins, proteins and carbohydrates were extracted and analyzed in each experiment in two growth phases (figure 9). The extractions were carried out according to a specific extraction plan, see appendix C.

3.4.1 Pigment analysis

For the pigment analysis samples of the 12 cultures were taken in each experiment in two stages of the growth phases. The first sample was taken when the culture was in its exponential growth phase. The second sample was taken in the end phase, to say in other words, when the cultures showed a decline in dry weight (figure 9).

In order to perform the chlorophyll and carotenoid extraction 5, 1 and 1 ml samples of each culture were taken for experiment 1, 2 and 3 respectively. Chlorophyll and carotenoids extraction was done by the methyl ethanol extraction method (Leu and Hsu, 2005). For full protocol see appendix D. The phycobilin pigments were extracted by a freeze-thawing process (Bennet & Bogorad, 1973 and Lawrenz et al., 2011), appendix E. A

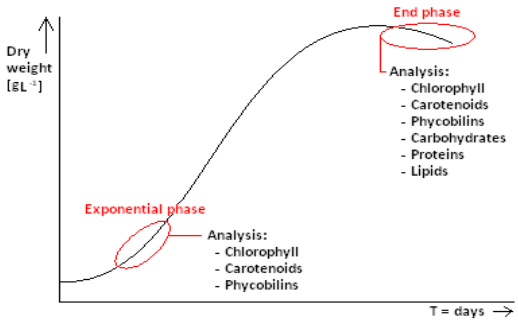

Figure 9*: Analysis per growth phase.*

sample of 5 ml was taken for this extraction from each culture in all the three experiments.

3.4.2 Protein and carbohydrate analysis

In order to determine the protein and the carbohydrate content of the cultivated *Rhodomonas sp.* samples were taken in the end phase of the cultivation process (figure 9).

For the protein determination 10, 5 and 5 ml of samples for each culture was taken for experiment 1, 2 and 3 respectively. Extraction was done according to the Folin reagent method (Lowry et. al., 1951), appendix F. The determination of the carbohydrates was done by an acid extraction method (DuBois et al., 1956). For each experiment 5 ml samples for each of the twelve cultures were taken, for full protocol see appendix G.

3.5 Statistical analysis

In order to compare the retrieved data a statistical analysis was carried out. A ANOVA and *t*-test was used to compare the cell densities, productivities, dry weights and the biochemical analysis results for the different light wavelengths in each experiment. In addition the results between the three different experiments were compared. The results shown are the mean (n=3) of the results per colour per experiment, where the error bars represents the standard deviation.

4. Results

4.1 Effect of low light conditions on the cultivation of *Rhodomonas sp.*

In the low light (LL) experiment *Rhodomonas sp.* was cultivated under a PFD of 8 ± 10 µmol m^2 s^{-1} and exposed to light colours blue, green, red and white. The cell density, productivity and dry weight of *Rhodomonas sp.* were monitored for eleven days, figure 10 and table 2. The LL experiment resulted in low dry weight and cell density. However, notable is that the cultures exposed to blue light resulted in a significant higher dry weight ($P < 0,05$), 0.30 ± 0.03 g/l for blue light, against 0.01 ± 0.002 and 0.06 ± 0.02 for red and green light respectively. In addition, the productivity for blue light was the most stable compared to the other colours (see appendix H). An exponential decrease in productivity can be observed from blue to white, table 2. Moreover, the cell density is 2.1, 2.4 and 2.8 fold lower for green, red and white respectively compared to blue.

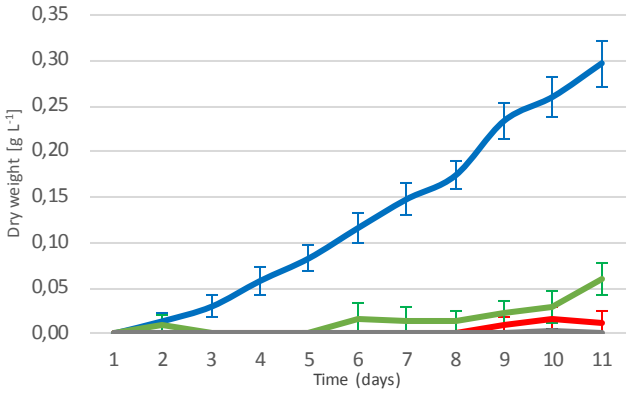

Figure 10: *Dry weight in the low light (LL) experiment for the cultures exposed to the wavelength colours blue, red, green and white (colours representing the lines) with a light intensity of 8 µmol m^2 s^{-1}. The error bars represent the standard deviation (n=3).*

Table 2*: The monitored parameters, maximum productivity (g L^{-1} day^{-1}), cell density (10^6 cells ml^{-1}) and protein level (pg $cell^{-1}$), are shown for the cultures exposed to the different wavelength of colours in the LL experiment. In descending order the productivity and cell density was the highest for blue> green > red> white.*

Wavelength color	Max. productivity (g L^{-1} day^{-1})	Cell density (10^6 cells ml^{-1})	proteins (pg $cell^{-1}$)
Blue	0,06 ± 0,020	2,23 ± 0,09	37 ± 1,53
Green	0,03 ± 0,010	1,06 ± 0,09	47 ± 1,09
Red	0,01 ± 0,001	0,91 ± 0,10	36 ± 7,46
White	0,001 ± 0,001	0,80 ± 0,03	48 ± 2,48

At two points in the LL experiment extractions were performed, at day 6 and day 11 (end of the experiment). Notable was that pigmentation (chlorophyll a (chl a) and b (chl b), carotenoids and phycoerythrin (PE)) increased considerably in the five days that lasted between the two extractions, figure 11. In the cultures that were exposed to blue and red light, chl a content doubled and a distinct increase in chl b was noticed in all the cultures, with 0.1 pg $cell^{-1}$ to 2.1 pg $cell^{-1}$ the highest increase at the green wavelength for chl b. The carotenoid content was close to zero at day 6, but reached up to 8.6 pg $cell^{-1}$, cultures exposed to red wavelengths, at the end of the experiment. The PE content increased slightly in the cultures exposed to red, green and white light, but almost doubled in the cultures exposed to blue light. Green and white light resulted in approximately the same protein level, the same occurs for the cultures exposed to red blue and red light, table 2.

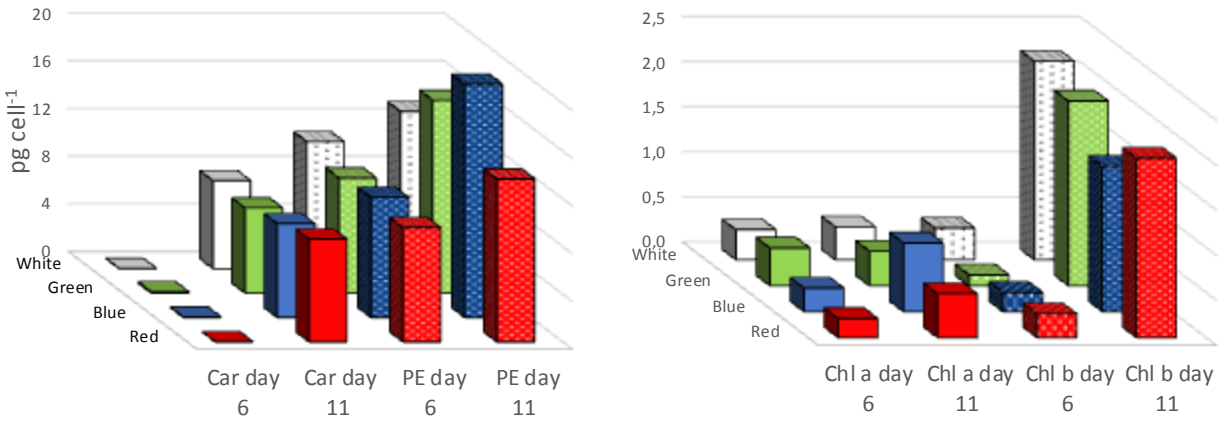

Figure 11: *Biochemical composition of Rhodomonas cultures exposed to wavelength colours red, blue, green and white at low light intensity (8 µmol m^2 s^{-1}). The plain coloured bars represents carotenoids (Car day 6, 11) and chlorophyll a (Chl a day 6, 11), where the dotted coloured bars represents phycoerythrin (PE day 6, 11) and chlorophyll b (Chl b day 6, 11) in pg $cell^{-1}$ at day 6 and day 11 of the cultivation period. This graph is a summary for the biochemical analysis in the low light experiment, for the extended graphs see appendix H.*

4.2 Effect of medium light conditions on the cultivation of *Rhodomonas sp.*

For medium light (ML) conditions *Rhodomonas sp.* cultures were cultivated under the same colours as for LL (blue, green, red and white) at a PFD of 60 ± 10 µmol m^2 s^{-1}. Figure 12 illustrates that the cultures which were illuminated by blue light showed the highest biomass, correlating with the results in LL conditions. A characteristic growth curve can be seen in figure 12 for all the cultures consisting of a lag phase, exponential phase, stationary phase and end phase. Blue light obtained quickly a considerable dry weight, which correlates with the high productivity (0.58 ± 0.01 day^{-1}) that was observed, table 3. The cultures exposed to the green light had a slightly higher dry weight in the first 7 days in respect to the red light. However, green and red followed the same stationary and end phase.

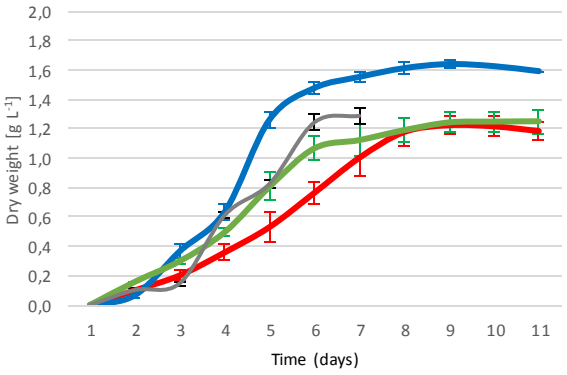

Figure 12*: Dry weight in the medium light (ML) experiment for the cultures exposed to the wavelength colours blue, red, green and white (colours representing the lines) with a light intensity of 60 ± 10 µmol m2 s-1. The error bars represent the standard deviation (n=3).*

Notable is that the cultures exposed to blue light, which resulted in the highest biomass, did not reached the highest cell density (4.91 ± 0.29 million cells/ml). The reference cultures (white light) exceeded the blue cultures and reached a cell density of 5.35 ± 0.16 million cells per ml, table 3. The lowest cell density was observed under green light, followed by red and blue. While green had the lowest cell density, the productivity was slightly higher than the productivity for the cultures exposed to red which exceeded the cell density of green with almost a million.

The highest protein level is found in the cultures exposed to the white light followed by green, red and blue. Notable is that the protein level for green and white is almost two times higher than the protein levels found in the cultures exposed to blue and red light. A significant higher carbohydrate level ($P < 0,05$) can be found in the cultures exposed to green light compared to the other colours. White light resulted in the lowest carbohydrate level.

Table 3: *The monitored parameters, maximum productivity (g L^{-1} day^{-1}), cell density (cells ml^{-1} 10^6), protein (pg $cell^{-1}$) and carbohydrates (pg $cell^{-1}$) levels, are shown for the cultures exposed to the different wavelength colours in the medium light (ML) experiment. In descending order the productivity was the highest for blue> white> green > red, where the cell density was the highest for white > blue > green > red.*

Wavelength colour	Productivity (g L^{-1} day^{-1})	Cell density (cells ml^{-1} 10^6)	proteins (pg $cell^{-1}$)	Carbohydrates (pg $cell^{-1}$)
Blue	0,58 ± 0,01	4,91 ± 0,29	235 ± 24,8	57 ± 1,0
Green	0,31 ± 0,04	2,97 ± 0,39	433 ± 14,8	88 ± 6,7
Red	0,24 ± 0,05	3,77 ± 0,03	251 ± 10,1	56 ± 0,8
White	0,47 ± 0,08	5,35 ± 0,16	496 ± 68,3	45 ± 0,9

Since there was a distinct exponential and end phase the biochemical extractions were performed in respect to these phases. Samples were taken at day 4 and 11 in the cultivation period, see figure 12. Where pigmentation increased during cultivation in the LL conditions, a reversed phenomenon occurred in the ML experiment. Chl a, chl b and the carotenoids decreased significantly ($P < 0.05$) from the exponential to the end phase for all the colours (blue, green, red), except for the white light, figure 13. The ML experiment did not resulted in a distinct PE concentration change between the exponential and end phase in the cultures exposed to red, green and white light, where only a slight decrease can be observed, figure 13. However, the cultures exposed to the blue light showed a diminution of 38% in respect to the exponential phase.

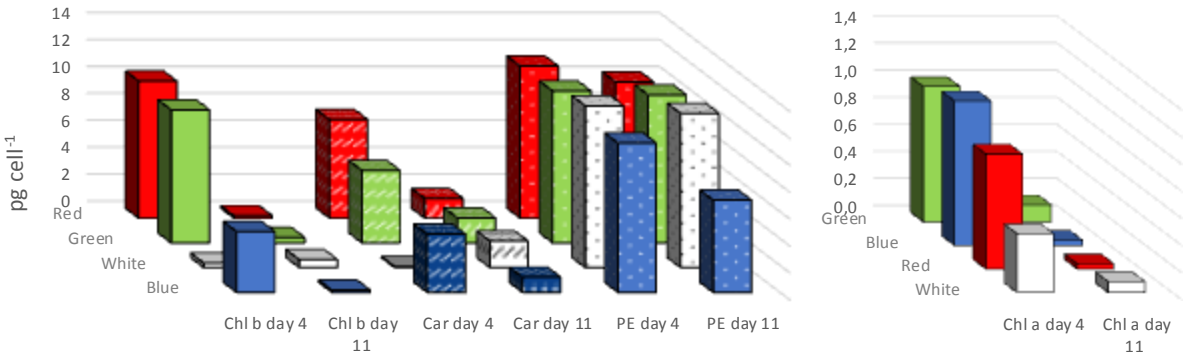

Figure 13: *Biochemical composition of Rhodomonas cultures exposed to wavelength colours red, blue, green and white at medium light intensity (60 µmol m^{-2} s^{-1}). The plain coloured bars represents chlorophyll b (Chl b day 4, 11) and chlorophyll a (Chl a day 4, 11), the dashed coloured bars represent carotenoids (Car day 4, 11) and the dotted coloured bars represent phycoerythrin (PE day 4, 11) in pg $cell^{-1}$ at day 4 and day 11 of the cultivation period for the corresponding light colours. This figure is a summary for the biochemical analysis in the medium light experiment, for extended graphs see appendix H.*

4.3 Effect of high light conditions on the cultivation of *Rhodomonas sp.*

The effects of cultivating *Rhodomonas sp.* under high light (HL) conditions with different wavelengths were examined by exposing the cultures to a PFD of 80 ± 20 µmol m^2 s^{-1} . Figure 14 shows the biomass of the cultures exposed to the different wavelengths. White and blue light resulted in the highest biomass level, 1.38 ± 0.08 and 1.39 ± 0.04 g L^{-1} respectively. However, the cultures grown under white light reached this level earlier in the cultivation period than the cultures grown under blue light. Notable is the characteristic growth curve of the cultures grown under white light and the significantly higher productivity compared to the other colors, table 4. The cultures exposed to red and green light follows the same pattern. However, in the end phase green shows an earlier descent in biomass than red. At day eight the dry weight of the cultures exposed to red light reaches the same level as blue.

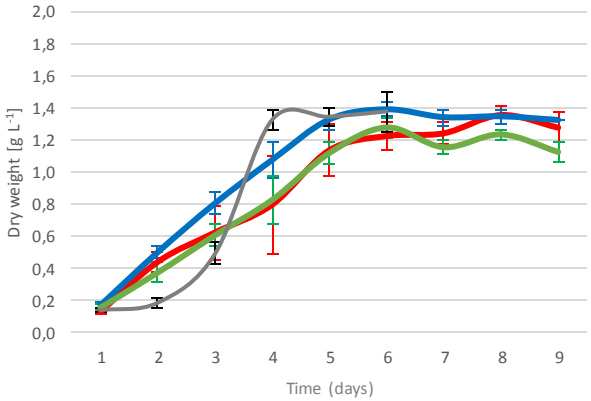

Figure 14: *Dry weight in the high light (HL) experiment for the cultures exposed to the wavelength colours blue, red, green and white (light colours representing the lines) with a light intensity of 80 ± 20 µmol m^2 s^{-1}. The error bars represent the standard deviation (n = 3).*

The highest protein level is found in the cultures exposed to red light followed by green, blue and white. In addition red light resulted in the highest carbohydrate level. The carbohydrates in the cultures exposed to green light is 30% lower than in red followed by blue (18%) and white (14%).

Table 4*: Monitored parameters, maximum productivity (g L^{-1} day^{-1}), cell density (10^6 cells ml^{-1}), protein (pg $cell^{-1}$) and carbohydrates (pg $cell^{-1}$) levels, are shown for the cultures exposed to the different wavelength colours in the high light (HL) experiment. In descending order the productivity was the highest for white > red > blue > green, where the cell density was the highest for white > blue > green > red.*

Wavelength color	Productivity (g L^{-1} day^{-1})	Cell density (10^6 cell ml^{-1})	proteins (pg $cell^{-1}$)	Carbohydrates (pg $cell^{-1}$)
Blue	0,33 ± 0,03	4,45 ± 0,15	118 ± 20,6	40 ± 2,9
Green	0,29 ± 0,09	4,43 ± 0,24	135 ± 8,50	34 ± 1,0
Red	0,34 ± 0,14	4,33 ± 0,07	161 ± 13,7	49 ± 1,7
White	0,53 ± 0,09	4,88 ± 0,22	111 ± 2,50	42 ± 0,1

The biochemical extractions in the HL experiment were, as with the ML experiment, performed according to the exponential and end phase. Therefore, samples were taken at day 4 and 9 respectively for green, blue and red. The samples for the analysis of the cultures exposed to white light were taken at day 3 and 5. Figure 16 shows the biochemical composition of the cultures grown under HL conditions. Notable is that pigmentation increases for chl a and carotenoids in all colors, except for white. The highest chl a content occurs in the cultures exposed to the white light (0,3 ± 0.04 pg $cell^{-1}$ in exponential phase), although red light results in the highest content of chl b (0.5 ± 0.07 pg $cell^{-1}$) and carotenoids (2.2 ± 0.08 pg $cell^{-1}$). PE, together with chl b, shows an significant decrease in pigments when the end phase is reached.

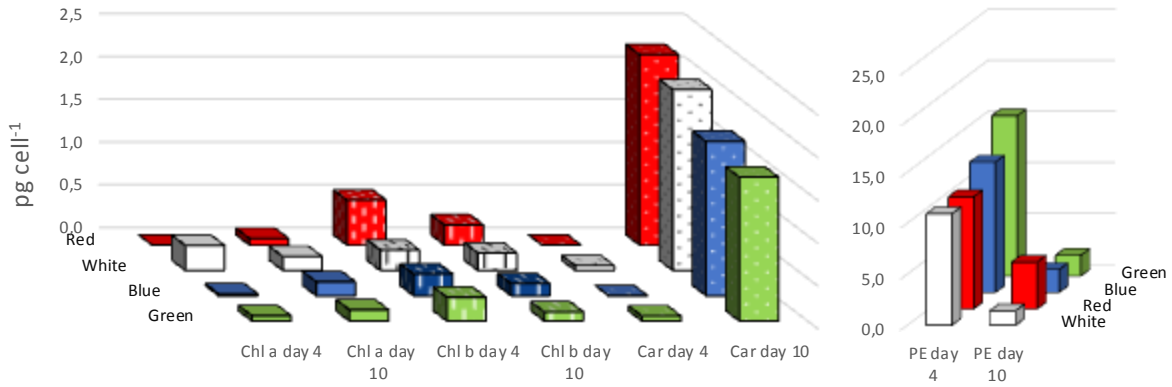

Figure 15: *Biochemical composition of Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white at high light (HL) intensity (80 µmol m^2 s^{-1}). The plain coloured bars represents chlorophyll a (Chl a day 4, 9) and phycoerythrin (PE day 4, 9), the striped coloured bars represent chlorophyll b (Chl b day 4, 9) and the dotted coloured bars represents carotenoids (Car day 4, 9) in pg $cell^{-1}$ at day 4 and day 9 of the cultivation period. This figure is a summary of the biochemical analysis for the high light experiment, for the extended graphs see appendix H.*

4.4 Comparison of low, medium and high light conditions

Distinct differences were observed between the LL, ML and HL conditions in respect to growth and pigmentation behaviour for cultivating *Rhodomonas sp.* under different wavelengths.

4.4.1 Growth: cell density, biomass and maximum productivity

When the cell density of the LL is compared to the ML experiment a significant growth ($P < 0,05$) for all colours can be seen, a 2.2, 2.8, 4.4 and 6.6 fold increase in cell density for blue, green, red and white respectively, table 5. However, when cell density results of ML is compared to the HL results a diminution takes place for the cultures grown under blue and white light (decrease of 464 and 898 thousand cells per ml respectively), although the cell density in the cultures exposed to red and green wavelengths are increasing with 1,4 and 0,6 million cells per ml.

Table 5: *Cell density (10^6 cells ml^{-1}) of the Rhodomonas sp. for low light (LL: 8 ± 10 µmol m^2 s^{-1}), medium light (ML: 60 ± 10 µmol m^2 s^{-1}) and high light (HL: 80 ± 20 µmol m^2 s^{-1}) conditions observed in this present study. The highest cell density for Rhodomonas sp. is reached in ML conditions by cultivating under white light.*

	Cell density [10^6 cells ml^{-1}]		
Wavelength colour	**LL conditions**	**ML conditions**	**HL conditions**
Blue	2,22 ± 0,09	4,91 ± 0,29	4,45 ± 0,15
Green	1,06 ± 0,09	2,97 ± 0,39	4,43 ± 0,24
Red	0,91 ± 0,10	3,77 ± 0,03	4,33 ± 0,07
White	0,80 ± 0,03	5,35 ± 0,16	4,88 ± 0,22

A significant increase of biomass (g L^{-1}) is observed ($P < 0.05$) when cultivation of *Rhodomonas sp.* is compared with LL to ML conditions for all colors, table 6. When ML is compared to HL conditions, HL resulted in a lower dry weight for the cultures exposed to blue light (diminution of 0.27 ± 0.04 g L^{-1}). However, a slight increase in dry weight was observed from ML to HL conditions for the cultures grown under red light (0.12 ± 0.06 g L^{-1}). The cultures exposed to green and white light did not show a distinct difference in dry weight when ML is compared to HL.

Table 6: *Observed biomass (dry weight g L^{-1}) of Rhodomonas sp. cultivated under low light (LL: 8 ± 10 µmol m^2 s^{-1}), medium light (ML: 60 ± 10 µmol m^2 s^{-1}) and high light (HL: 80 ± 20 µmol m^2 s^{-1}) conditions exposed to different light colours The highest Rhodomonas sp. biomass is reached under ML conditions when exposed to blue light.*

	Dry weight [g L^{-1}]		
Wavelength colour	**LL conditions**	**ML conditions**	**HL conditions**
Blue	0,30 ± 0,03	1,64 ± 0,04	1,39 ± 0,04
Green	0,06 ± 0,02	1,25 ± 0,08	1,28 ± 0,06
Red	0,02 ± 0,01	1,23 ± 0,06	1,35 ± 0,05
White	0,01 ± 0,01	1,29 ± 0,06	1,38 ± 0,08

However, the cultures grown under white light in the HL experiment showed notable behavior according to the dry weight and productivity. Where for the cultures exposed to blue, green and red light no distinct lag phase is visible, white light is showing the perfect characteristic growth curve for algae, see figure 15. Moreover, a considerably high productivity (0.53 ± 0.09 g L^{-1}) was observed under high light conditions and the cell density exceeds the other colors with 438 ± 221 thousand cells. Notable however is when cell density of the white light cultures under HL conditions is compared to ML an decrease of 469 ± 221 thousand cells is observed.

These observations indicates that white light under HL conditions will give the highest productivity (0.53 ± 0.09 g L^{-1} day^{-1}) compared to the other colors with the same conditions. However, when the productivity is compared to ML, white light does not exceed the productivity and dry weight level of blue light (white HL: 0.53 ± 0.09 g L^{-1} day^{-1} against blue ML: 0.58 ± 0.01 g L^{-1} day^{-1} for productivity and white HL: 1.38 ± 0.12 g L^{-1} against blue ML: 1.64 ± 0.03 g L^{-1} for dry weight), see table 7. In general ML conditions are more favorable for cultivating *Rhodomonas sp.* than HL conditions when looked at these parameters.

Table 7: *Overview of dry weight, cell density and maximum productivity for the cultures exposed to white and blue light in the low light (8 ± 10 µmol m^2 s^{-1}), medium light (60 ± 10 µmol m^2 s^{-1}) and high light (80 ± 20 µmol m^2 s^{-1}) experiment. Italic fond numbers shows the highest value within the group.*

	Dry weight		Cell density		Max. productivity	
	Blue [g L^{-1}]	**White [g L^{-1}]**	**Blue [10^6 cells ml^{-1}]**	**White [10^6 cells ml^{-1}]**	**Blue [g L^{-1} day^{-1}]**	**White [g L^{-1} day^{-1}]**
Low light	0,30 ± 0.03	0,01 ± 0,01	2,22 ± 0,09	0,80 ± 0,03	0,06 ± 0,02	0,01 ± 0,01
Medium light	*1,64 ± 0,03*	1,29 ± 0,05	4,91 ± 0,29	*5,35 ± 0,16*	*0,58 ± 0,01*	0,47 ± 0,14
High light	1,39 ± 0,04	1,38 ± 0,12	4,45 ± 0,15	4,88 ± 0,22	0,33 ± 0,03	0,53 ± 0,09

4.4.2 Biochemical composition

In respect to the biochemical composition of *Rhodomonas sp.* cultivated under the different light intensities and wavelengths, distinct differences were observed for pigmentation, carbohydrate and protein levels.

4.4.2.1 Chlorophyll a content

An increase for chl a is observed from LL to ML experiment in the exponential phase for all the colours, table 8. Chlorophyll a content in the cultures exposed to blue light increased 3.6 fold from 0.3 ± 0.02 to 1.1 ± 0.06 pg cell^{-1}. White showed the lowest increase in pigments, only a 1.3 fold increase, followed by green and red. However, where pigmentation increased in the exponential phase from LL to ML almost a 100% diminution was noticed when ML is compared to HL for all colours except for white. White light is only showing a diminution of 33% compared to HL.

In the end phase of the cultures a decrease of pigmentation is noted when LL and ML is compared. However a slight increase is observed from ML to HL for all colours.

Table 8: *Chlorophyll a content in Rhodomonas sp. cultures in low light (LL: 8 ± 10 µmol m^2 s^{-1}), medium light (ML: 60 ± 10 µmol m^2 s^{-1}) and high light (HL: 80 ± 20 µmol m^2 s^{-1}) conditions for blue, green, red and white light in the exponential and end phase in pg cell^{-1}.*

	Chl a content exponential phase [pg cell^{-1}]			Chl a content end phase [pg cell^{-1}]		
Wavelength Colour	**LL conditions**	**ML conditions**	**HL conditions**	**LL conditions**	**ML conditions**	**HL conditions**
Blue	0.3 ± 0.02	1.1 ± 0.06	0.0 ± 0.00	0.8 ± 0.13	0.0 ± 0.00	0.2 ± 0.01
Green	0.4 ± 0.19	1.0 ± 0.02	0.1 ± 0.05	0.4 ± 0.04	0.1 ± 0.05	0.1 ± 0.02
Red	0.2 ± 0.05	0.8 ± 0.06	0.0 ± 0.00	0.5 ± 0.20	0.0 ± 0.00	0.1 ± 0.02
White	0.3 ± 0.06	0.4 ± 0.04	0.1 ± 0.04	0.4 ± 0.02	0.1 ± 0.01	0.2 ± 0.02

4.4.2.2 Chlorophyll b content

In respect to the exponential phase, chl b content increases for all colors expect white (diminution of 30%) when LL is compared to ML, table 9. The largest increase was seen under green light, almost a 100 fold increase compared to LL. When ML is compared to HL a diminution takes place for all the colors and the chl b content is reaching approximately the same levels as under LL conditions.

When looked at chl b content in the end phase of the cultures a diminution can be observed from LL to HL in all colors.

Table 9: *Chlorophyll b content in Rhodomonas sp. cultures in low light (LL: 8 ± 10 µmol m^2 s^{-1}), medium light (ML: 60 ± 10 µmol m^2 s^{-1}) and high light (HL: 80 ± 20 µmol m^2 s^{-1}) conditions for blue, green, red and white light in the exponential and end phase in pg cell^{-1}.*

	Chl b content exponential phase [pg cell^{-1}]			Chl b content end phase [pg cell^{-1}]		
Wavelength Colour	**LL conditions**	**ML conditions**	**HL conditions**	**LL conditions**	**ML conditions**	**HL conditions**
Blue	0.2 ± 0.03	4.5 ± 0.01	0.3 ± 0.07	1.6 ± 0.18	0.2 ± 0.05	0.1 ± 0.02
Green	0.1 ± 0.06	9.9 ± 2.84	0.3 ± 0.02	2.1 ± 0.02	0.4 ± 0.07	0.1 ± 0.01
Red	0.3 ± 0.08	10.2 ± 1.02	0.5 ± 0.07	2.0 ± 0.22	0.2 ± 0.02	0.2 ± 0.04
White	0.4 ± 0.07	0.3 ± 0.03	0.2 ± 0.02	2.2 ± 0.17	0.6 ± 0.66	0.2 ± 0.05

4.4.2.3 Carotenoids content

If looked at the carotenoids content in the exponential phase under LL conditions almost no pigmentation is observed, table 10. However, when the cultures are exposed to ML conditions a massive increase in pigmentation takes place for all the colours except for white. Notable is that when the cultures are exposed to HL conditions pigmentation again decreases to almost zero.

In the end phase a diminution of carotenoids is observed when LL is compared to ML for all colours. The highest diminution is found in the cultures exposed to blue light (7 fold decrease), where the cultures exposed to green, white and red a 4, 4 and 6 fold decrease was noticed. Notable is that a slight increase of carotenoids in all colours takes place when ML is compared to HL, except for the cultures exposed to green light. Blue and red showed the highest increase from ML to HL (0.7 pg cell^{-1}).

Table 10: *Carotenoids content in Rhodomonas sp. cultures in low light (LL: 8 ± 10 µmol m^2 s^{-1}), medium light (ML: 60 ± 10 µmol m^2 s^{-1}) and high light (HL: 80 ± 20 µmol m^2 s^{-1}) conditions for blue, green, red and white light in exponential and end phase in pg cell^{-1}.*

	Carotenoids content exponential phase [pg cell^{-1}]			Carotenoids content end phase [pg cell^{-1}]		
Wavelength colour	**LL Conditions**	**ML conditions**	**HL conditions**	**LL conditions**	**ML conditions**	**HL conditions**
Blue	0.1 ± 0.02	4.3 ± 0.28	0.0 ± 0.00	7.9 ± 0.90	1.1 ± 0.16	1.8 ± 0.05
Green	0.1 ± 0.06	5.4 ± 0.54	0.1 ± 0.04	7.1 ± 0.20	1.9 ± 0.34	1.7 ± 0.07
Red	0.0 ± 0.00	7.3 ± 0.50	0.0 ± 0.00	8.6 ± 1.86	1.5 ± 0.39	2.2 ± 0.39
White	0.0 ± 0.00	0.1 ± 0.01	0.1 ± 0.01	7.4 ± 0.06	2.0 ± 0.18	2.1 ± 0.08

4.4.2.4 Phycoerythrin content

When PE content is compared between the three different experiments a massive loss of pigments can be seen from LL to HL conditions. ML showed a decrease of 32, 26 and 10% for green, red and white respectively when compared to LL, see table 11. Notable is the high PE diminution in cultures exposed to blue light from LL to ML. A diminution of almost 90% takes place for each color when LL is compared to HL conditions, except for the cultures exposed to the red light, table 12.

Table 11: *Phycoerythrin (PE) content for the low and medium light experiment (LL: 8 ± 10 µmol m^2 s^{-1}, ML: 60 ± 10 µmol m^2 s^{-1}) at the end of the experiments for the cultures exposed to the light wavelengths blue, red, green and white light in pg cell^{-1}. The diminution in PE between the LL and ML experiment is shown in percentages. Highest diminution is found under blue light, 64%.*

Wavelength colour	**PE content LL [pg cell^{-1}]**	**PE content ML [pg cell^{-1}]**	**PE diminution [%]**
Blue	19,6 ± 1,11	6.90 ± 0,90	-64
Green	16,1 ± 1,00	11.0 ± 1,00	-32
Red	13,7 ± 1,75	10,1 ± 1,70	-26
White	13,2 ± 0,58	11,5 ± 0,35	-10

Table 12: *Phycoerythrin (PE) content for the low and high light experiment (LL: 8 ± 10 µmol m^2 s^{-1}, HL: 80 ± 20 µmol m^2 s^{-1}) at the end of the experiments for the cultures exposed to the light wavelengths blue, red, green and white light in pg cell^{-1}. The diminution in PE between the LL and HL experiment is shown in percentages, all except for red have a PE loss of >85%.*

Wavelength color	**PE content LL [pg cell^{-1}]**	**PE content HL [pg cell^{-1}]**	**PE diminution [%]**
Blue	19,6 ± 1,11	2,2 ± 0,64	-89
Green	16,1 ± 1,00	2,0 ± 0,74	-88
Red	13,7 ± 1,75	4,6 ± 0,24	-66
White	13,2 ± 0,58	1,4 ± 0,06	-89

4.4.2.5 Carbohydrate content

Carbohydrate content decreases from ML to HL in all the cultures exposed to the different colours, table 13. The lowest diminution can be observed in white light, only 7%. However, the cultures exposed to green light in HL conditions showed the largest diminution, 61% when compared to ML, followed by blue and red.

***Table 13**: Carbohydrate content for the medium and high light (ML: 60 ± 10 µmol m^2 s^{-1}, HL: 80 ± 20 µmol m^2 s^{-1}) at the end of the experiments for the cultures exposed to the light wavelengths blue, red, green and white light in pg $cell^{-1}$. The diminution in carbohydrate content between the ML and HL experiment is shown in percentages.*

Wavelength colour	Carbohydrate content ML [pg $cell^{-1}$]	Carbohydrate content HL [pg $cell^{-1}$]	Carbohydrate diminution [%]
Blue	57 ± 6.7	40 ± 1,0	-30
Green	88 ± 0.8	34 ± 1.7	-61
Red	56 ± 1.0	49 ± 2.9	-13
White	45 ± 0,9	42 ± 0,1	-7

4.4.2.6 Protein content

When the protein content of LL is compared to ML an increase in all colours can be observed, table 14. A 6, 7, 9 and 10 folded increase takes place for the cultures exposed to blue, red, green and white respectively. However, when the ML conditions are compared to the HL conditions the protein levels decreases. The largest diminution can be observed under white light, 4.5 fold decrease from ML to HL.

***Table 14:** Protein content for the low, medium and high light experiment (LL: 8 ± 10 µmol m^2 s^{-1}, ML: 60 ± 10 µmol m^2 s^{-1}, HL: 80 ± 20 µmol m^2 s^{-1}) at the end of the experiments for the cultures exposed to the light wavelengths blue, red, green and white light in pg $cell^{-1}$.*

	Protein content [pg $cell^{-1}$]		
Wavelength colour	LL conditions	ML conditions	HL conditions
Blue	37 ± 1.5	235 ± 26	118 ± 21
Green	47 ± 1.1	433 ± 15	135 ± 9.0
Red	36 ± 7.5	251 ± 1.0	161 ± 14
White	48 ± 2.5	496 ± 68	111 ± 3.0

5. Discussion

5.1 Growth

The cultures in the LL experiment resulted in low dry weight, productivity and cell density. This observation is in correlation with previous studies, which reveals that light saturation for *Rhodomonas sp.* occurs around a PFD of 60 µmol m^2 s^{-1} and therefore irradiance lower than this will cause a suboptimal growth (Bartual et al., 2002; Hammer et al., 2002; Chaloub et. al., 2014; Thi Thuy Vu et. al., 2015). However, blue light resulted in a significant higher ($P < 0,05$) dry weight and cell density in the LL experiment compared to the other colors. This can be explained by the absorption spectra of the photosynthetic pigments (figure 5). The blue LED's emits at a wavelength where phycoerythrin, chlorophyll b and carotenoids can effectively take up photons resulting in a more efficient photon transfer to the reaction centre (Mirkovic, 2009). In addition, blue photons contains distinctly more energy (40%) than red light which has effect on the excited state of chlorophyll (Matthijs et. al. 1995).

Where a diminution of cell density is observed from ML to HL in the cultures exposed to blue and white, an increase was noticed for green and red light. This might be explained by the fact that red and green wavelengths contain less energy than blue and white light (which contains blue, green and red) and therefore need a higher intensity to obtain the same photosynthetic efficiency resulting in higher cell density (Matthijs et al., 1996). However, the cell density in HL of green and red light did not exceed the cell density in ML of blue and white.

5.2 Pigmentation

In the LL experiment an increase in pigmentation in all colours from exponential to end phase is observed. These results suggests that low light conditions increases the efficiency of cells in an algal culture to capture photons for photosynthesis, which is in correlation with the study of Thinh (1983). The LED lights used in the LL experiment were low in capacity resulting in low light intensity. As stated in the study of Chaloub et. al. (2014) low light intensity trigger algal cells to synthesize more pigments, considering cultivation with LED lights with low capacity and therefore resulting in low light intensity, as useful for pigment production for biochemical purposes. The study of Rhiel et. al. (1985) showed that the chloroplast of the *Cryptomonas maculata* under low light conditions (1.28 W m^{-2}) contains 50% more of the cell area (longitudinal cross section) than a cell exposed to high light conditions (4,42 W m^{-2}). In other words, more pigments are found in a chloroplast under LL conditions. There must be taken in account though that to reach this density and biochemical composition the cultivation period is quite long and the amount of energy needed might be higher than shorter cultivation periods with higher intensities.

The results of Chaloub et. al. (2014) reveals that the highest phycoerythrin content (14 pg $cell^{-1}$) for *Rhodomonas sp*. occurs at a PFD of 15 µmol m^2 s^{-1} (20 °C) with an implementation of a light:dark cycle. This finding is consistent with the observations in this present study for the PE content in the cultures exposed to white light under LL conditions (13.2 ± 0.58 pg $cell^{-1}$). However, the cultures exposed to blue wavelengths in LL showed a content of 19.6 ± 1.11 pg $cell^{-1}$ PE, significant higher ($P<0.05$) than the content of PE for white light.

Pigmentation behaviour under ML conditions were the opposite from the LL conditions. Where pigmentation increased from exponential to end phase in LL, a reversed phenomenon occurred in ML. This can partly be explained by photo acclimation which occurs in response to PFD and spectral density changes (Falkowski & LaRoche, 1991) and PFD saturation (Chaloub et al., 2015).

However, another process should be taken in account when looked at the biochemical composition in the end phase of the cultures. The cultures in the present study, as well for the ML and HL experiment, showed a change in colour from red to yellow/greenish when stationary and end phase was reached, figure 16. When such transition in colour occurs it indicates nitrogen starvation (da Silva et. al., 2009). The study of Bartual et. al. (2002) and da Silva et al. (2009) reveals a massive loss of chlorophylls and a distinct loss of PE under nitrogen starvation, in addition a loss in soluble proteins was observed. These findings might support the assumption that nutrients became a limiting factor in this present study for the ML and HL experiments.

Figure 16: *ML cultures exposed to red wavelengths reaching the end phase. A change of colour can be seen from red to yellow.*

When nitrogen starvation occurs, PE can function as a source of amino acids for the synthesis of new proteins or protein turnover (Rhiel et al., 1985; da Silva et. al., 2009). However, no distinct difference in ML cultures between PE content was observed when the exponential and end phase is compared. Only blue light resulted in a distinct diminution (38%) in respect to exponential phase. One explanation for this phenomenon can be the fact that the cultures exposed to the blue light reached the stationary phase the earliest caused by a rapid productivity compared to the cultures exposed to the other colours. In addition, colour transition was observed the earliest of all cultures during the cultivation period indicating early nitrogen starvation in the cultures exposed to blue light. Lafarga-De la Cruz et al. (2006) states that the reduction of pigment content is considered as a process of auto regulation of the photosynthetic apparatus to reach a balance between the gain of light and the demand of energy necessary for micro algae growth, suggesting that energy in the blue cultures was used for growth and not for pigment synthesis.

In the HL experiment a loss of pigments in the end phase was observed for chl b and PE. This can be explained by the fact that a diminution of growth is occurring and therefore less pigmentation is necessary (LaFarga-De La Cruz et. al., 2005). In addition, as mentioned previously, the algal cultures showed a transition of color when stationary and end phase was reached, suggesting nitrogen starvation (da Silva et al., 2009). It is a common response for algae to decrease their pigment content when nutrient deficiency occurs (Thi Thuy Vu et al., 2016).

In the study of Rhiel et. al. (1985) the morphology of the thylakoids in cells exposed to low and high light (1.28 W m^{-2} and 4,42 W m^{-2} respectively) were investigated. It revealed that 90% of the phycoerythrin was lost in the cells exposed to the high light (Rhiel et al., 1985). The results of this present study shows the same phenomenon, up to 89 % of PE is lost when LL is compared too high light, table 12. However, PE diminution in the HL conditions for the cultures exposed to red wavelengths is considerably lower than the PE decrease for blue, green and white. This can be explained by the fact that the red wavelengths are not well absorbed by the pigments thus more pigments are needed to capture the photons and reach the same photosynthetic activity as for the other wavelengths. This in correlation with observations by (Mohsenpour et al., 2012) where *C. vulgaris* adapted its pigmentation to the change of wavelength conditions.

5.3 Proteins and carbohydrates

The protein level is significantly higher in the ML experiment compared to the LL experiment, suggesting that the optimal PFD range for protein production is 60 µmol m^2 s^{-1}. The study of Falkowski & LaRoche (1991) states that when irradiance is switched from high to low light, macromolecule biosynthesis initially is diverted from lipids and carbohydrates to proteins. Since stock cultures were grown under a PFD of 120 µmol m^2 s^{-1} and then were exposed to ML conditions, the observation of Falkowski and LaRoche (1991) might support the high protein level for the *Rhodomonas sp.* in ML conditions. However, further study is necessary to investigate this phenomena.

According to da Silva et. al. (2009) carbohydrates in *Rhodomonas sp.* can function as a storage by forming carbon skeletons or as starch (Fernandes et. al., 2017). To this carbon skeletons nitrogen can be added, observed by a distinct increase of carbohydrates under nitrogen limited conditions (150 pg ce ll^{-1} for nutrient excess against 25 pg cell^{-1} under nitrogen sufficient conditions) (da Silva et al., 2009; Fernandes et. al., 2017). The observation for the white light (44 ± 0.9 pg cell^{-1}) is in accordance with previous results obtained by Fernández-Reiriz et al. (1989) for carbohydrates which is 40,6 pg cell^{-1} in the late stationary phase.

6 Conclusion

In this study the effect of low, medium and high irradiance levels on the cultivation of Rhodomonas sp. with different wavelengths representing the colours red, blue, green and white was investigated. There investigated which wavelength and intensity would result in the best quantity and quality of *Rhodomonas sp*. defined by dry weight, productivity, cell density, chlorophyll a, chlorophyll b, carotenoids, phycoerythrin, carbohydrate and protein content.

Blue light resulted in the highest biomass of *Rhodomonas sp.* in low light (LL), medium light (ML) and high light (HL) conditions, where the highest biomass was found under ML conditions, resulting in 1.64 g L^{-1}. Moreover, blue light resulted in the best productivity under LL and ML conditions, where ML conditions were observed to be the highest, 0.58 ± 0.01 g L^{-1} day^{-1}. On the other hand, white light showed the highest productivity, 0.53 ± 0.09 g L^{-1} under HL conditions. The cell density was observed to be the highest in ML conditions for white light, 5.35 ± 0.16 million cells ml^{-1}. In addition, white light resulted in the highest cell density in HL conditions, however blue gave the best result under LL and ML.

In respect to pigmentation the highest values of PE and carotenoids were found under LL conditions in the end phase of *Rhodomonas sp.* cultures, resulting in 19.6 ± 1.12 pg $cell^{-1}$ and 8.6 ± 1.85 pg $cell^{-1}$ for phycoerythrin and carotenoids exposed to blue and red light respectively. For chlorophyll a and b ML conditions resulted in the highest levels in the exponential growth phase for all the colours. Moreover, blue and red light were observed to result in the highest chlorophyll a (1.1 ± 0.06 pg $cell^{-1}$) and b (10.2 ± 1.02 pg $cell^{-1}$) content respectively. The composition of carbohydrates and proteins resulted to be overall the highest in ML conditions for respectively green (88 ± 0.82 pg $cell^{-1}$) and white light (459 ± 68 pg cell $^{-}$1) giving the highest content.

This study has demonstrated that cultivating *Rhodomonas sp.* with an irradiance of 60 µmol m^2 s^{-1} under blue wavelengths of light will result in the best overall growth defined by highest biomass and maximum productivity, and will result in the highest chlorophyll a content. In addition, the highest chlorophyll b, carbohydrate and protein levels are found under the same light intensity for the colours red, green and white respectively. Phycoerythrin and carotenoids content will be the highest when *Rhodomonas sp.* is cultivated with a PFD of 8 µmol m^2 s^{-1} with blue and red light respectively.

7 Recommendations

This study recommends to cultivate *Rhodomonas sp.* under a PFD of 60 µmol m^2 s^{-1} with blue light to achieve the highest biomass , maximum productivity, protein and carbohydrate level. In order to get the highest pigmentation a PFD of 8 µmol m^2 s^{-1} is recommended with blue or red wavelengths depending on the pigment. However, further study is necessary to carbohydrate and protein level behaviour in *Rhodomonas sp.* in respect to the effects of light wavelength and intensity, and in respect of the growth phases.

This study could be elaborated by finding the perfect PFD for pigmentation. Interesting could be to investigate pigmentation at a wavelength of 525nm (green) and 570nm (yellow) for the absorption peak in PE in a range of LL conditions compared to the blue wavelengths used in this study. For Chlorophyll a and b further study is recommended at ML conditions with eye on the absorption peaks of these pigments. In addition, investigation to carotenoids in HL conditions and the role of these pigments for the *Rhodomonas sp*., especially ß-carotene, might be interesting for carotenoid production since literature suggest that it is a form of photo protection (Vu et al., 2016).

In this present study there is looked at wavelengths representing the colours blue, green and red with white as reference. Cultivating *Rhodomonas sp.* with a combination of these colours, especially red/blue for pigmentation and blue/white for growth, is recommended to investigate further, since the combination of these colours might induce pigmentation and growth even more positively. Other colour combinations, green/blue, blue/yellow or a combination of three LED strips with each another colour could give interesting results. In addition, changing the colours from exponential to end phase is recommended as further study for pigmentation and growth behaviour for *Rhodomonas sp.* Blue light could be used in the early growth phases for biomass production where red light in later growth phases can induce pigmentation. Another important aspect in cultivating algae in respect to pigmentation and growth is implementing a light – dark cycle. According to literature (Chaloub et al., 2015) this will have a positive effect on pigmentation and growth for *Rhodomonas sp*. when implemented under LL and ML conditions.

Since the experimental setup in this present study caused fluctuations in intensity between the individual cultivation flaks an improved construction is recommended. This could be done by making one construction per flask, meaning one LED strip surrounding one cultivation flask and therefore having the ability to give each culture the same PFD. Another option could be the use of combined LED/-driver integrated circuit strips. With these strips, in combination with for example Arduino hardware, there is the ability to control each LED light on the strip separately in respect to intensity and colour (Wishkerman & Wishkerman, 2017), although basic knowledge of programming is required when this technique is used. Another aspect that might be interesting to take a look at is the energy use of LED lights, where cultivation for pigmentation (LL) and growth (ML) can be compared on energy use against efficiency and expected harvest.

At last up scaling of cultivating *Rhodomonas sp.* in photo bioreactors or continuous systems is recommended to see if pigmentation and growth behaviour under the recommended conditions give the same results.

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9 List of appendices

Appendix A: L1 medium

L1
Medium

Guillard and Hargraves (1993) - please see note at the bottom of this page

This enriched seawater medium is based upon f/2 medium (Guillard and Ryther 1962) but has additional trace metals. It is a general-purpose marine medium for growing coastal algae.

To prepare, begin with 950 mL of filtered natural seawater. Add the quantity of each component as indicated below, and then bring the final volume to 1 litre using filtered natural seawater. The trace element solution and vitamin solutions are given below.

Autoclave.

Final pH should be 8.0 to 8.2.

Table 15: *The different components of the L1 medium with their quantity and molar concentration.*

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75.00 g L^{-1} dH_2O	1 mL	8.82×10^{-4} M
$NaH_2PO_4 \cdot H_2O$	5.00 g L^{-1} dH_2O	1 mL	3.62×10^{-5} M
$Na_2SiO_3 \cdot 9 H_2O$	30.00 g L^{-1} dH_2O	1 mL	1.06×10^{-4} M
trace element solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5mL	---

L1 Trace Element Solution

To 950 mL dH2O add the following components and bring final volume to 1 litre with dH2O. Autoclave.

Table 16: *Components of the trace element solution for the L1 medium.*

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na2EDTA · 2H2O	---	4.36 g	1.17×10^{-5} M
FeCl3 · 6H2O	---	3.15 g	1.17×10^{-5} M
MnCl2·4 H2O	178.10 g L^{-1} dH2O	1 mL	9.09×10^{-7} M
ZnSO4 · 7H2O	23.00 g L^{-1} dH2O	1 mL	8.00×10^{-8} M
CoCl2 · 6H2O	11.90 g L^{-1} dH2O	1 mL	5.00×10^{-8} M
CuSO4 · 5H2O	2.50 g L^{-1} dH2O	1 mL	1.00×10^{-8} M
Na2MoO4 · 2H2O	19.9 g L^{-1} dH2O	1 mL	8.22×10^{-8} M
H2SeO3	1.29 g L^{-1} dH2O	1 mL	1.00×10^{-8} M
NiSO4 · 6H2O	2.63 g L^{-1} dH2O	1 mL	1.00×10^{-8} M
Na3V04	1.84 g L^{-1} dH2O	1 mL	1.00×10^{-8} M
K2CrO4	1.94 g L^{-1} dH2O	1 mL	1.00×10^{-8} M

f/2 Vitamin Solution

(Guillard and Ryther 1962, Guillard 1975)

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH2O, dissolve the thiamine, add the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 litre with dH2O. At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

Table 17: *Components of the vitamin solution for the L1 medium.*

Component	Primary Stock	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	0.1g L^{-1} dH2O	10 mL	2.05×10^{-9} M
cyanocobalamin (vit. B12)	1.0 g L^{-1}dH2O	1 mL	3.69×10^{-10} M

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26- 60. In Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.

Guillard, R.R.L. and Hargraves, P.E. 1993. Stichochrysis immobilis is a diatom, not a chrysophyte. Phycologia ***32:*** *234-236.*

Guillard, R.R.L. and Ryther, J.H. 1962. Studies of marine planktonic diatoms.

I. Cyclotella nana Hustedt and Detonula confervacea Cleve. Can. J. Microbiol. ***8:*** *229-239*.

Please note:

In April 2013, it was brought to our attention that the recipe for L1 medium on the NCMA website differs from the original publication of L1 medium(Guillard, R. R. L., and P. E. Hargraves. "Stichochrysis immobilis is a diatom, not a chrysophyte." Phycologia 32.3 (1993): 234-236). The intended final concentration for K2CrO4 was 10^{-9} molar. There is an inconsistency in the 1993 paper as the directions for making the chromium stock solution that are in that paper result in a 10^{-8} molar final concentration in the medium. We have been using those directions for making our L1 trace metal mix for almost 15 years, resulting in a final concentration of 10^{-8}molar. We plan to continue to do so as a matter of consistency.

This error was corrected by Guillard in 2003. The corrected recipe can be found in the Manual on Harmful Marine Microalgae, Edited by G.M. Hallegraeff, D.M. Anderson and A.D. Cembella, Technical director: H.O. Enevoldsen, Oceanographic Methodology series, 2nd revised edition, 2003, 978-92-3-103871-6, UNESCO Publishing. Note that this L1 recipe uses a different vanadium compound (ammonium metavanadate, NH_4VO_3) to make the vanadium stock solution than the 1993 paper, which used sodium orthovanadate, Na_3VO_4. According to Sigma Aldrich, the concentration of vanadium ionic species depends upon the pH and redox potential of the environment. Sodium orthovanadate is more soluble in water than ammonium metavanadate. The concentration of vanadium in both recipes is more or less the same. Since the concentration of vanadium in seawater is $1.0\text{-}4.0 \times 10^{-8}$ molar with an average of 3.2×10^{-8} molar (Abbasse, Ghiasse, Baghdad Ouddane, and Jean Fischer. "Determination of trace levels of dissolved vanadium in seawater by use of synthetic complexing agents and inductively coupled plasma–atomic emission spectroscopy (ICP–AES)." Analytical and bioanalytical chemistry 374.5 (2002): 873-878), the L1 trace metal addition of vanadium should not elevate the concentration of vanadium in L1 medium much above that of natural seawater.

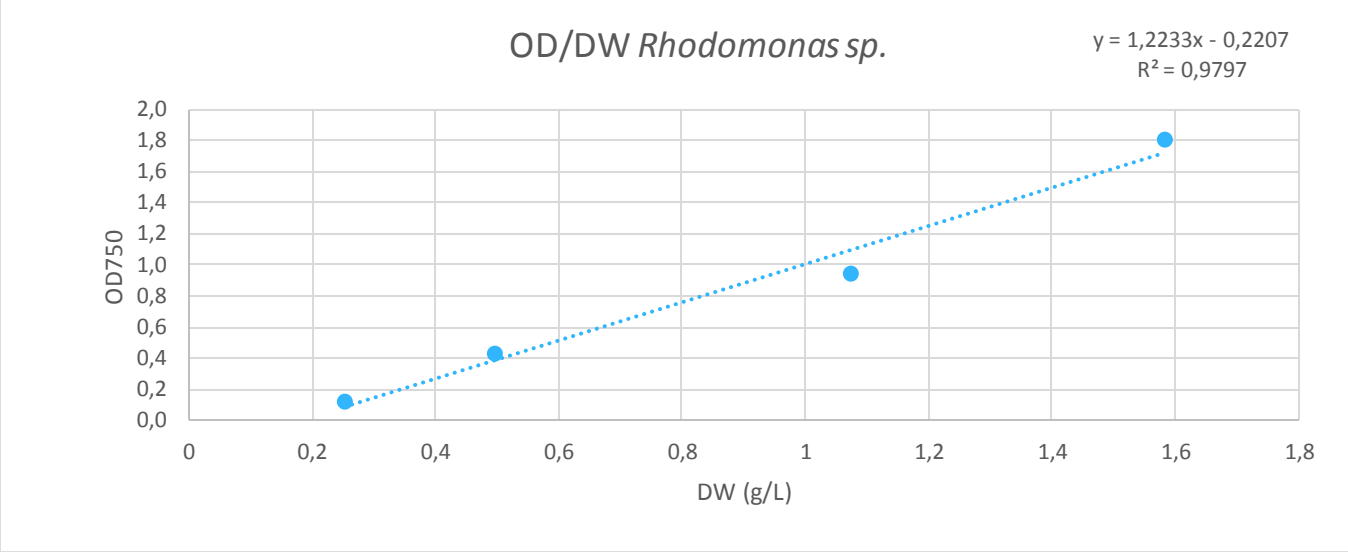
Appendix B: Dry weight measurements *Rhodomonas sp.*

Carried out by C. Latsos prior to the research.

Samples of the *Rhodomonas sp.* strain is diluted 2, 5 and 10 times in order to find the optical density and the corresponding dry weight to generate a curve, see graph 3. Next, the samples (5 ml) were filtered, weight and dried in the oven overnight at 80°C. The formula from the curve can be used to calculate the dry weight when the optical density of the *Rhodomonas sp.* culture is measured, without performing the dry weight measures for every sample.

Table 18: *Dry weight measurements of Rhodomonas sp. strain.*

Sample dilution	Sample [ml]	Filter [#]	Empty [gr]	Filter + sample [gr]	Dry weight [mg/ml]	Optical Density [750nm]
1/1	5	1	2,1917	2,2007	1,8054	1,584
1/2	5	2	2,1900	2,1947	0,9428	1,076
1/5	5	3	2,1930	2,1951	0,4213	0,498
1/10	5	4	2,1992	2,1998	0,1204	0,253



Graph 1: *Measured dry weight plotted against the measured optical density for the Rhodomonas sp.*

Appendix C: Extraction plan

Materials:

- 5 ml pipet + tips
- 1 ml pipet + tips
- Gloves
- 5 tube racks
    - 3 large pink
    - 1 small green
    - Carton rack for big tubes
- 60 plastic sample tubes
    - 45 x 15 ml tubes (Chlorophyll, proteins, phycobilins, lipids)
    - 15 x 50 ml tubes (Lipids, carbohydrates)
- 48 glass tubes for supernatant
- 12 small Erlenmeyer's (100 ml)
- Demi water bottle
- Waste beaker, demi water beaker
- Extraction solutions
    - Phosphate buffer
    - Methyl ethanol
    - Solution A – E
    - Phenol solution
- Ear phones and good music

Method:

1. Take samples and centrifuge for 15 minutes at 4350 rpm
    a. Phycobilins → 5 ml
    b. Protein → 5 ml
    c. Carbohydrates → 5 ml
    d. Chlorophyll → 1 ml
    e. Lipids → Depending on dry weight of cultures
2. Mix the extraction solutions and refill (solution D, folin reagent)
3. Label tubes and racks
4. Carbohydrates
    a. Add 2 ml of H2O
    b. Add 2 ml of phenol reagent
    c. 40 minutes rest (TIMER!)
5. Chlorophyll
    a. Add 5 ml methanol 100%
    b. 10 minutes ultrasound bath (TIMER!)
6. Phycobilins
    a. 5 ml phosphate buffer
    b. Homogenize
7. Chlorophyll samples in heat bath 50 minutes (TIMER!)
8. Homogenize phycobilins and store in freezer (note time!)

9. Carbohydrates
    a. Add 10 ml sulphuric acid
    b. 10 minutes rest at room temperature
    c. Homogenize samples
    d. Centrifuge 10 minutes at 4350 rpm
10. Protein
    a. Add 5 ml solution D
    b. Rest for 10 minutes (TIMER!)
    c. Add 0,5 ml solution E
    d. Rest for 30 minutes (TIMER!)
11. Chlorophyll in fridge for 15 minutes (TIMER!)
    a. Centrifuge for 8 minutes at 4350 rpm
12. Centrifuge protein 10 minutes at 3000 rpm
13. Read the absorbance of all the samples in the sea lab
14. Remove phycobilins from the freezer after 24 hours and put in fridge
15. After another 24 hours centrifuge phycobilins for 15 minutes at 4350 rpm
16. Read the absorbance of phycobilins in the sea lab

Appendix D: Protocol for chlorophyll and total carotenoids determination

(Adapted by: María Cuaresma/Jeroen de Vree from Leu and Hsu, 2005)

Procedure:

1. Take 1ml of culture (depending on its density will be needed more or less sample volume) and put it in a 15ml plastic vial.
2. Centrifuge at 4400rpm during 8 min at 4ºC
3. Add 5ml of methanol 100% to the pellet
4. Put 10 min in ultrasound bath to disregard the pellet with methanol
5. Incubate the cell suspension at 60ºC during 50min
6. Incubate the cell suspension at 0ºC during 15 min
7. Centrifuge the suspension at 4400rpm during 8 min
8. Add more methanol if the pellet is not white after centrifugation and repeat the extraction
9. Measure chlorophyll and carotenoid content in a spectrophotometer at 470nm, 652nm and 665nm in a quartz cuvette (blank done with methanol)

Calculations:

10. Use Arnon's equations to determine the chlorophyll and carotenoid content:

$$Chl_a = (16.72 \cdot A_{665.2} - 9.16 \cdot A_{652.4}) \cdot \text{dilution factor}$$

$$Chl_b = (34.09 \cdot A_{652.4} - 15{,}28 \cdot A_{665.2}) \cdot \text{dilution factor}$$

$$Chl_{tot} = Chl_a + Chl_b$$

$$Carot_{tot} = \frac{\text{dilution factor} \cdot 1000 \cdot A_{470} - 1.63 \cdot Chl_a - 104.96 \cdot Chl_b}{221}$$

Arnon's equation based on: Liechtenthaler: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes (1987). Methods on enzymology 148: 350-382

Temperature shock based on: Leu, K.L., Hsu, B.D.: A programmed cell disintegration of Chlorella after heat stress. (2005). Plant Science, 168: 145-152

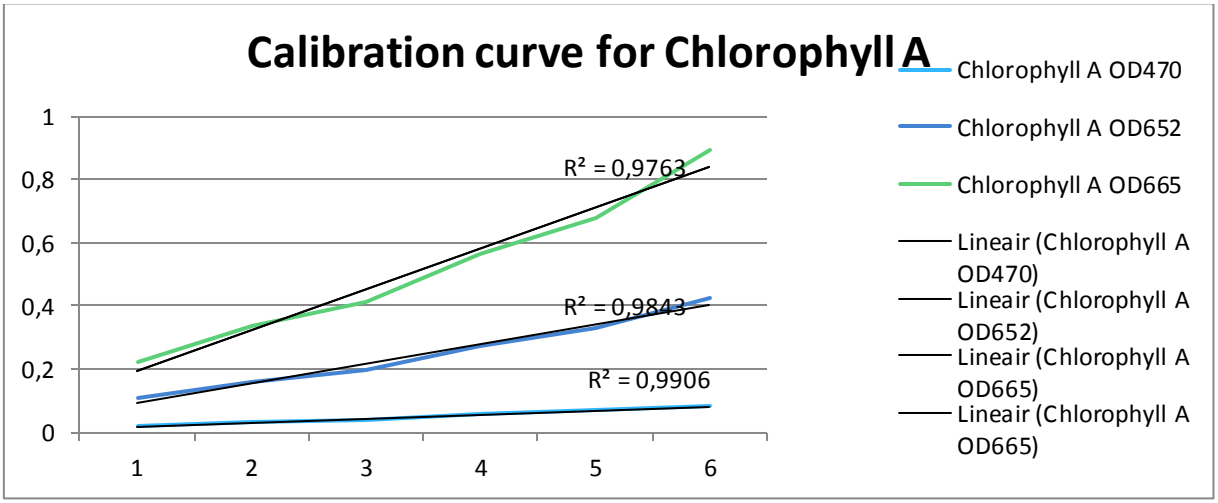
Chlorophyll-a calibration curve

After the experiments done, the last remaining thing was to make the calibration curve. For this the Chlorophyll A was dissolved in methanol in a known concentration (1 mg/mL). This was further diluted to keep within the linear range of the spectrophotometer. The dilutions made and measured for this are shown in table 2.

Chlorophyll A conc. (gr/L)	OD470	OD652	OD665
5.5556	0.021	0.106	0.220
11.111	0.030	0.157	0.337
16.667	0.040	0.199	0.409
22.222	0.055	0.273	0.567
27.778	0.067	0.329	0.676
33.333	0.083	0.423	0.895

The differences in the absorption at the differing can be explained, by the wavelength at which chlorophyll results in an extinction. At a wavelength of 470 nm chlorophyll should not result in an extinction as this is the region where carotenoids result in an extinction. It can also be seen in the table that almost no extinction can be seen at the wavelength of 470 nm. Chlorophyll does create an extinction at 665 nm and also a minor extinction at 652 can be seen, last is about 50% of the extinction at 665 nm.

The calibration curves are shown in graph 3.



Graph 2: *Calibration curve for chlorophyll A*

It can be seen that for all the wavelengths a linear correlation can be found between the concentration and extinction. All lines had a R^2 of above 0.95, this means that the calibration line was made in a reliable way.

Appendix E: Protocol for the estimation of phycobiliproteins

(adapted from Bennet & Bogorad, 1973 and Lawrenz et al., 2011)

Estimation of Phycobiliproteins

1. 0.05M Phosphate buffer (containing equal volumes of 0.1 M K_2HPO_4 and KH_2PO_4) is prepared. To prepare 0.1M K_2HPO_4, 1.741 g is added to 100 ml distilled water. For 0.1 M KH_2PO_4, 1.36 g is added to 100 ml distilled water. (Rather 2.72 g KH_2PO_4 to 200 ml d.w.)
2. Equal volume of K_2HPO_4 and KH_2PO_4 are added the pH is set to 6.7 **by adding KH_2PO_4 to K_2HPO_4**.
3. Now cryptophytic mass is centrifuged @ 3000-4000 rpm for 10-15 minutes.
4. Pellet is suspended into 3 ml or 5 ml newly prepared 0.05M Phosphate buffer and kept in **-80°C for 24-48 h** in plastic screw-capped tubes. (Avoid use of glassware's during freezing @ -80°C)
5. After this step it is kept at **below 5 °C for 24-36 h** for thawing. This process may be repeated once (in case of thick sheathed cyanobacteria).
6. Cryptophytic suspension is now centrifuged and supernatant is taken up for taking absorbance **@ 545 nm**. Take 0.05M Phosphate buffer as blank.

0.1M K_2HPO_4 = **1.741** g K_2HPO_4 to **100 ml** d.w.

0.1 M KH_2PO_4 = **2.72** g KH_2PO_4 to **200 ml** d.w.

Calculations:

$$c = \frac{A}{\varepsilon d} * MW$$

Where:

C = Phycobilin concentration

A = Absorbance

ε = Molar extinction coefficient (Phycoerythrin: 2.41 * 10^6 L mol^{-1} cm^{-1})

d = Path length of cuvette

MW = Molecular weight of phycobilins (Phycoerythrin: 240.000 g/mol)

Bennett, A., & Bogorad, L. (1973). COMPLEMENTARY CHROMATIC ADAPTATION IN A FILAMENTOUS BLUE-GREEN ALGA. The Journal of Cell Biology, 58(2), 419–435.

Lawrenz, E., Fedewa, E. J., & Richardson, T. L. (2011). Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. Journal of Applied Phycology, 23(5), 865–871. https://doi.org/10.1007/s10811-010-9600-0

Appendix F: Protocol for the protein determination

(adapted from Lowry et al., 1951)

Reagents:

- SOLUTION A: 2% of Na_2CO_3 in 0.1N NaOH (4g/L)

  (conservation period of 15 days)

- SOLUTION B: 0.5% of $CuSO_4 \cdot 5H_2O$ in water

  (conservation period of 1 month)0

- SOLUTION C: TARTRATE of K or Na 1%

  (conservation period of 1 month)

- SOLUTION D: 50 mL of A+1 mL of B+1 mL of C

  (conservation period of 1 day)

- SOLUTION E: Folin reagent diluted 2 times (1 vol. Folin+1 vol. H_2O

  (conservation period of 1 day)

Stock solution:

Prepare a stock solution at a concentration of 100 µg/mL of SIGMA beef albumin.

Make 5 dilutions in order to have 5 different concentrations:

Table 19: *Dilution scheme for the stock solution.*

[µg/mL]	Stock solution (mL)	H_2O (mL)
100	4	0
75	3	1
50	2	2
25	1	3
0	0	4

Materials:

1. Glass tubes of at least 15 mL that can be centrifuged;
2. Centrifuge;
3. Spectrophotometer;

Methodology:

1. Put the filter in a numbered tube. In case of the stock dilutions add 1 mL of each dilution in a different numbered tube.
2. Add 5 mL of Solution D and homogenize.
3. Let the tubes rest for 10 minutes at room temperature.
4. Add 0.5 mL of Solution E and homogenize the sample.
5. Let the tubes rest for 30 minutes at room temperature.
6. Centrifuge at 3000r/min (10 minutes).
7. Transfer the supernatant into a new tube and read the tubes at 750 nm.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265-275.

Appendix G: Protocol for the carbohydrates determination

(adapted from DuBois et al., 1956)

Reagents:

1. Phenol solution 5% in water

   (conservation period of 15 days, in the dark at 4° C because phenol oxidizes very quickly)

2. Sulphuric acid (H_2SO_4)

Stock solution:

1. Prepare a stock solution at a concentration of 100 µg/mL of glucose sigma. Make 5 dilutions in order to have 5 different concentrations:

Table 9: Dilution scheme for the stock solution.

[µg/mL]	Stock solution (mL)	H_2O (mL)
100	4	0
75	3	1
50	2	2
25	1	3
0	0	4

Materials:

1. Glass tubes of at least 15 mL that can be centrifuged;
2. Centrifuge;
3. Spectrophotometer;

Methodology:

1. Put the filter in a numbered tube. In case of the stock dilutions add 1 mL of each dilution in a different numbered tube.
2. Add 2 mL of H_2O to the tubes with filters.
3. Add 2 mL of the phenol solution in all tubes and homogenize.
4. Let the tubes rest for 40 minutes at room temperature.
5. Rapidly add 10 mL of H_2SO_4 (BE CAREFULL, EXOTERMIC REACTION) and homogenize the sample.
6. Let the tubes rest for 10 minutes at room temperature.
7. Centrifuge at 5000r/min (10 minutes).
8. Transfer the supernatant into a new tube and read the tubes at 490 nm.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28, 350-356.

Appendix H: Experimental data

List of figures experimental data:

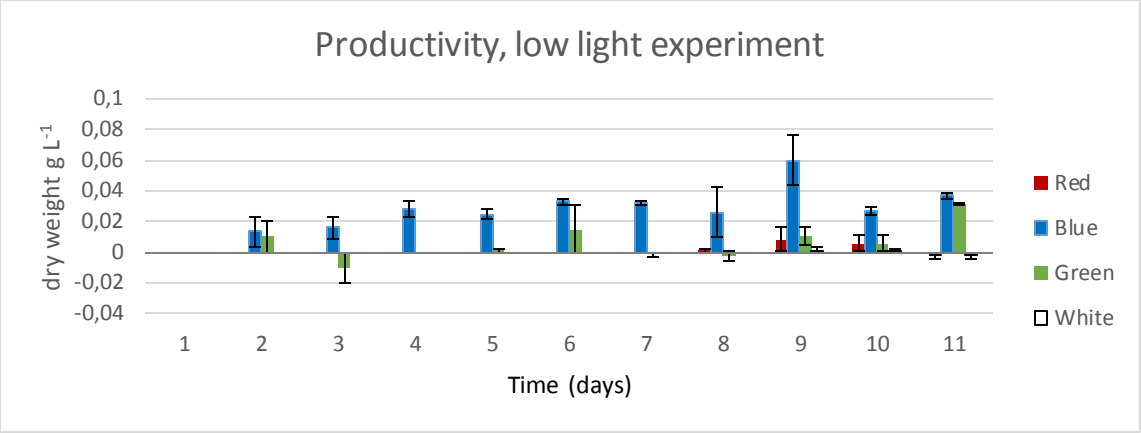


***Figure 17**: Productivity of Rhodomonas sp. cultures in the low light experiment (8 ± 10 µmol m^2 sec^{-1}) for the wavelength colours red, blue, green and white in g L^{-1} day^{-1}.*

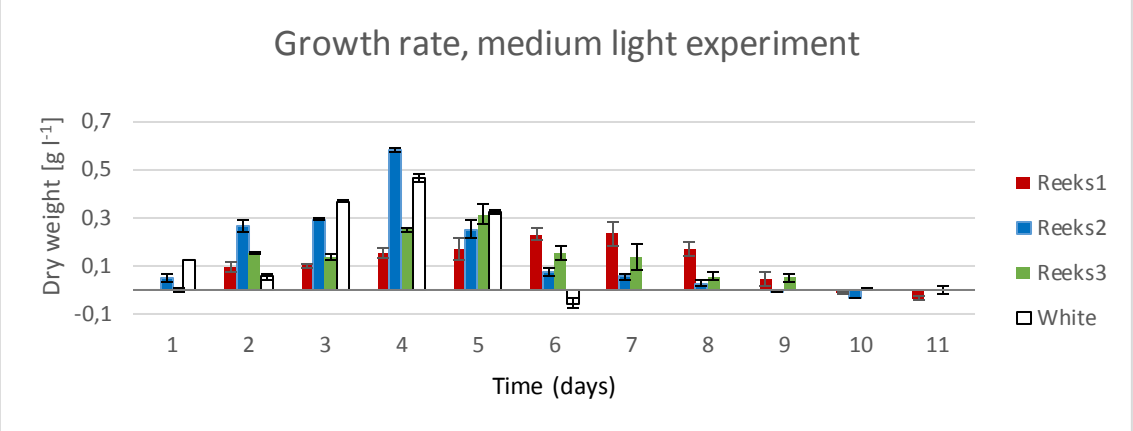


***Figure 18:** Productivity of Rhodomonas sp. cultures in the medium light experiment (60 ± 10 µmol m^2 sec^{-1}) for the wavelength colours red, blue, green and white in g L^{-1} day^{-1}.*

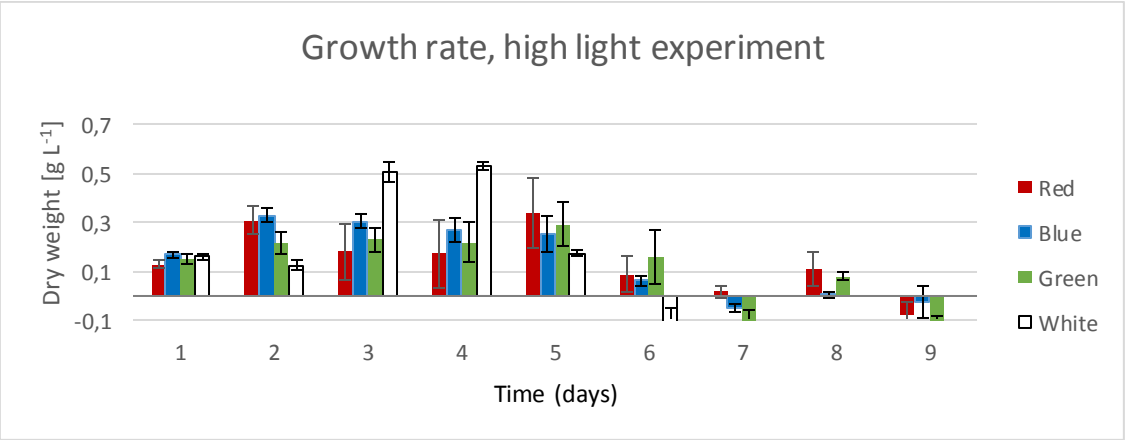


***Figure 19:** Productivity of Rhodomonas sp. cultures in the high light experiment (80 ± 10 µmol m^2 sec^{-1}) for the wavelength colours red, blue, green and white in g L^{-1} day^{-1}.*

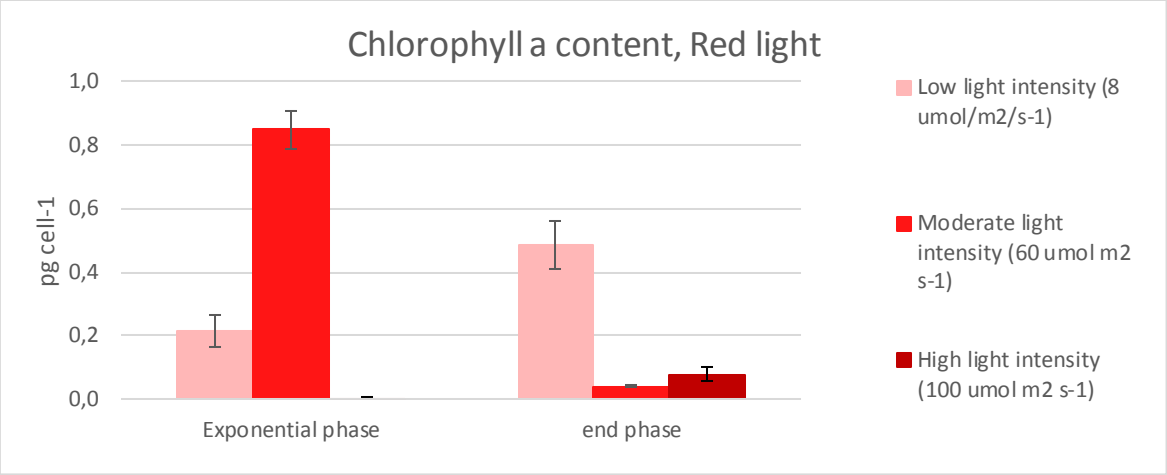


Figure 20: *Chlorophyll a content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under low light, medium light and high light conditions.*

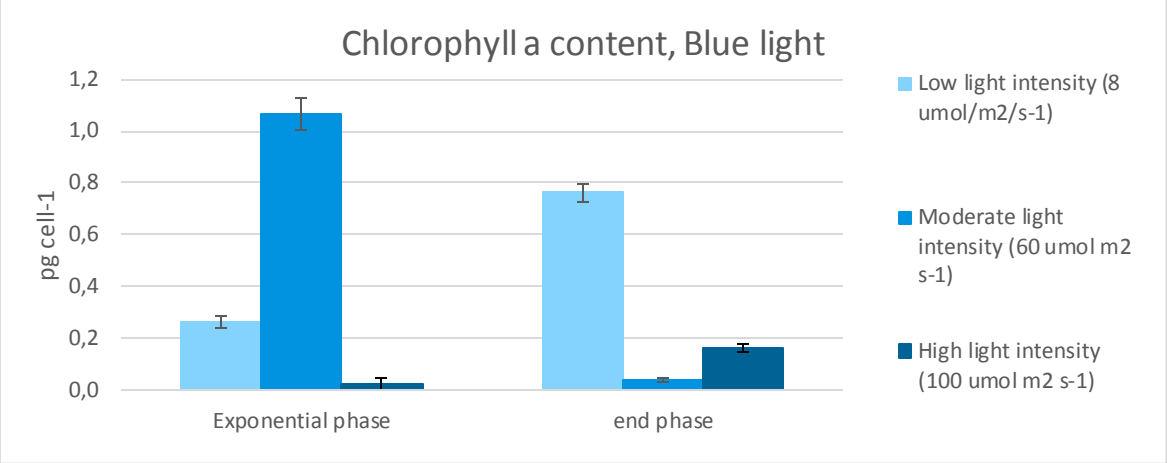


Figure 21: *Chlorophyll a content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under low light, medium light and high light conditions.*

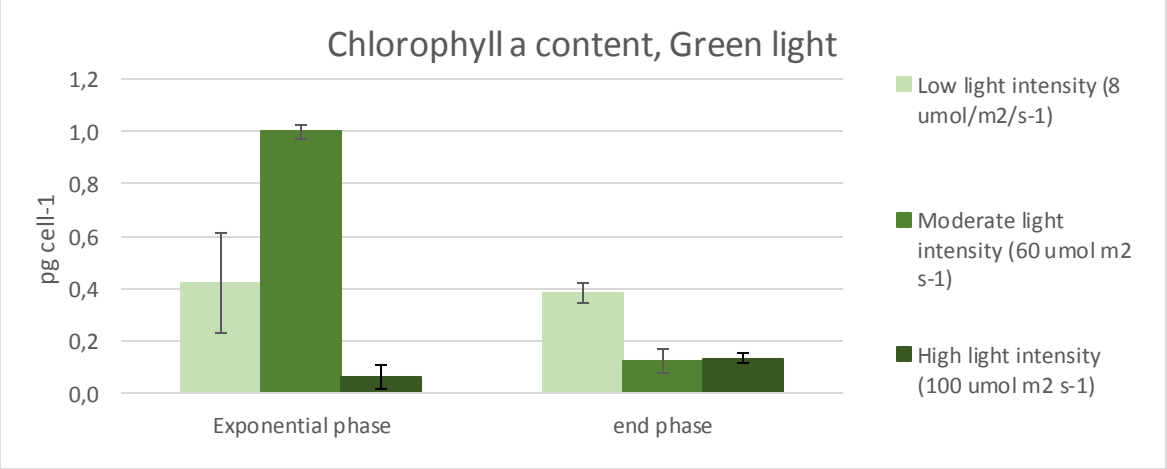


Figure 22: *Chlorophyll a content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under low light, medium light and high light conditions.*

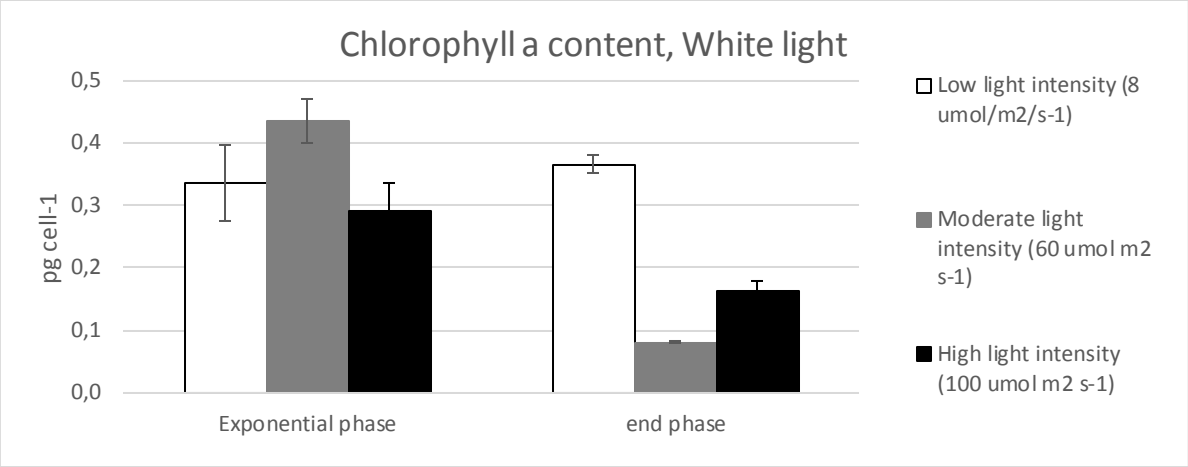


Figure 23: *Chlorophyll a content in Rhodomonas sp. cultures exposed to white light (λ_{range}: 415 - 720 nm) under low light, medium light and high light conditions.*

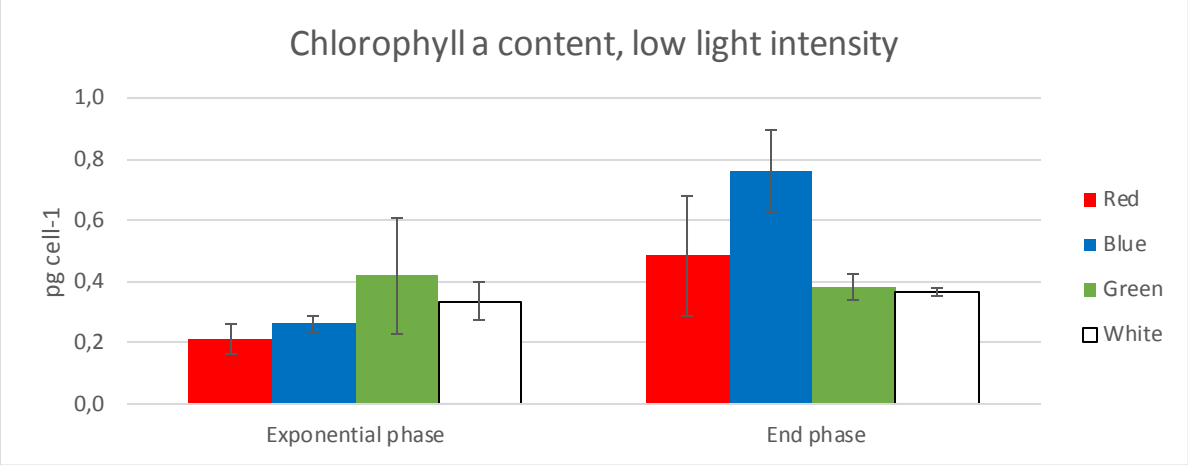


Figure 24: *Chlorophyll a content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under low light conditions (8 ± 10 µmol m^2 sec^{-1}).*

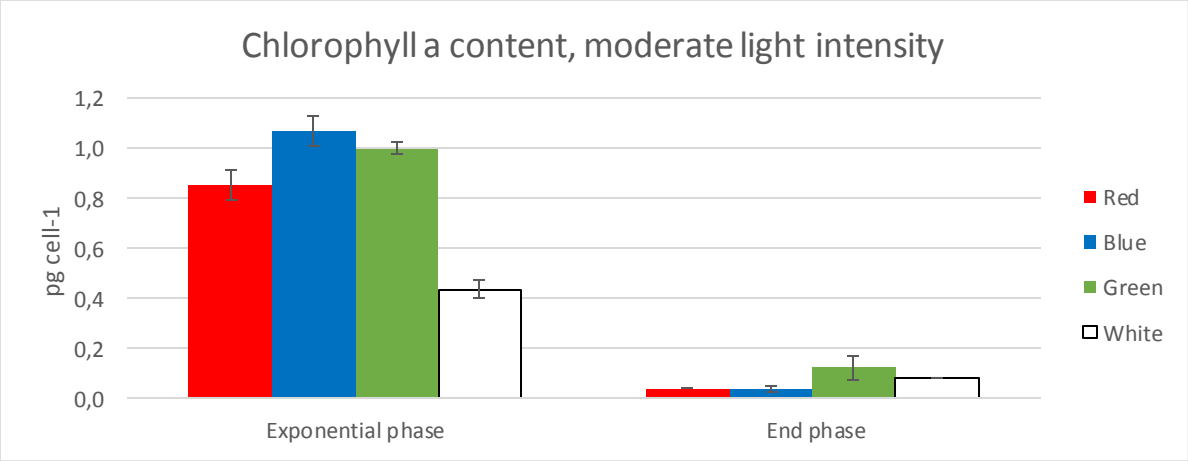


Figure 25: *Chlorophyll a content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

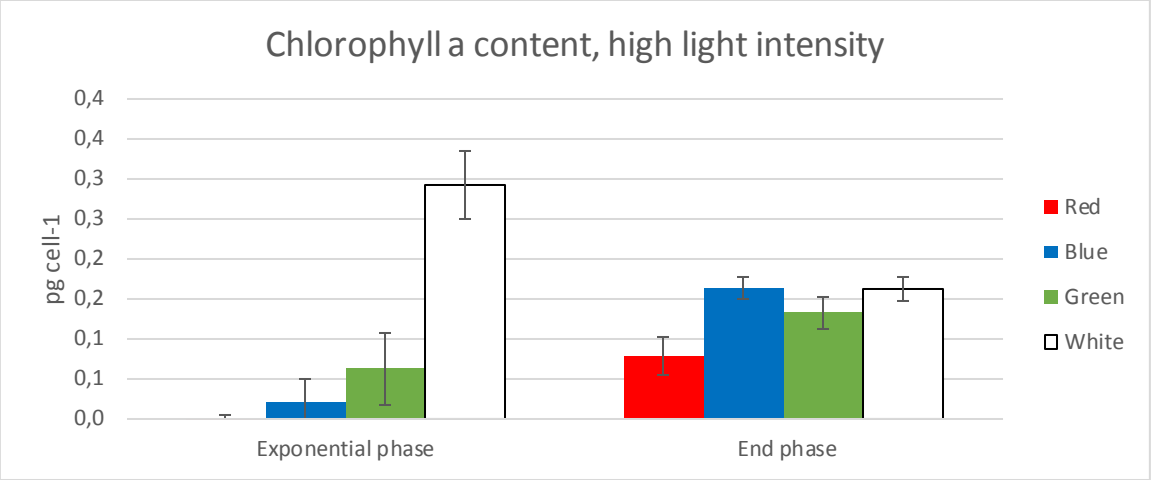


Figure 26: *Chlorophyll a content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 10 µmol m^2 sec^{-1}).*

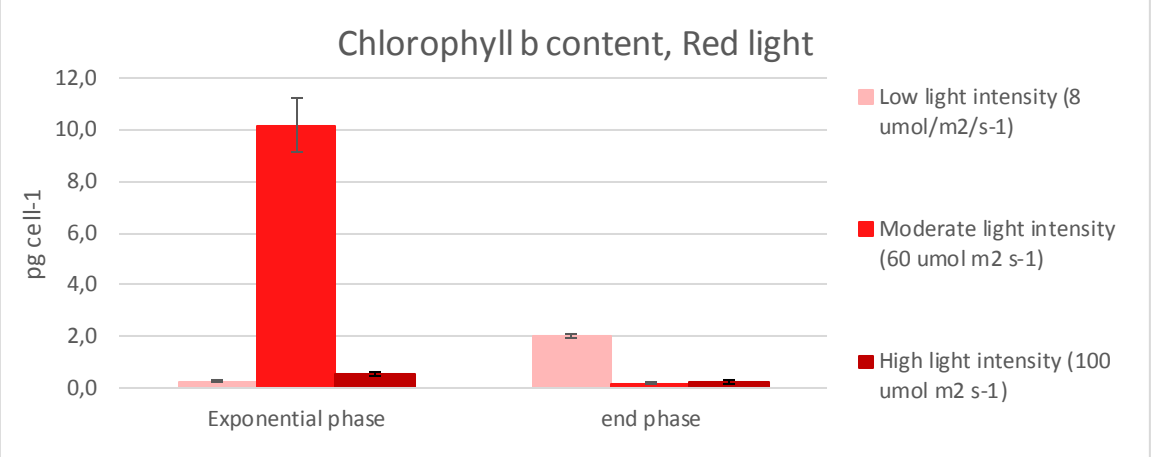


Figure 27: *Chlorophyll b content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under low light, medium light and high light conditions.*

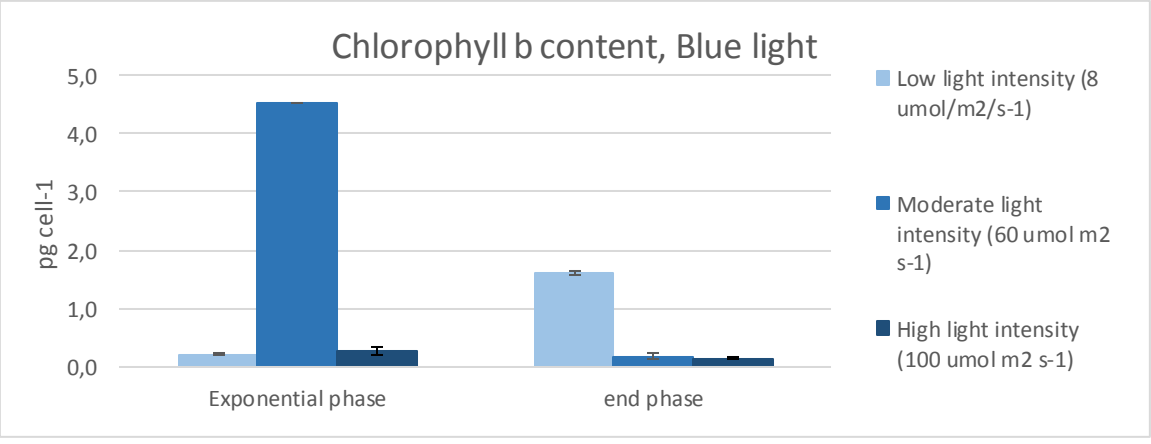


Figure 28: *Chlorophyll b content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under low light, medium light and high light conditions.*

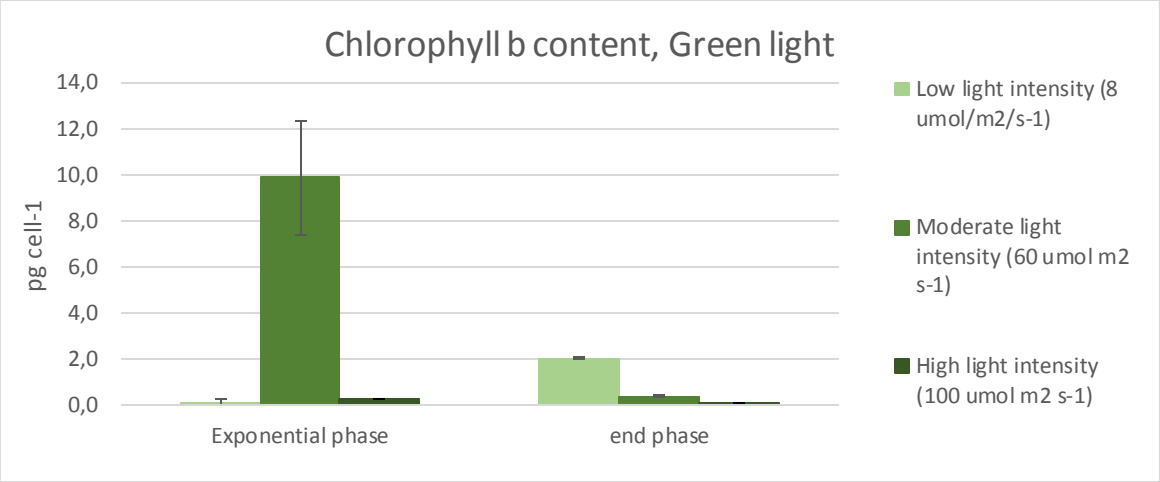


Figure 29: *Chlorophyll b content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under low light, medium light and high light conditions.*

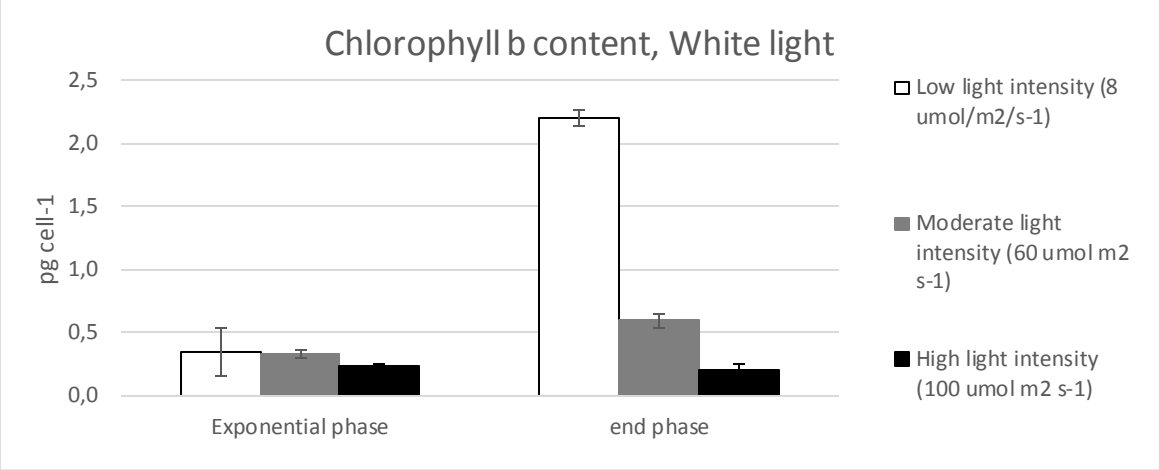


Figure 30: *Chlorophyll b content in Rhodomonas sp. cultures exposed to white light (λ_{range}: 417 - 720 nm) under low light, medium light and high light conditions.*

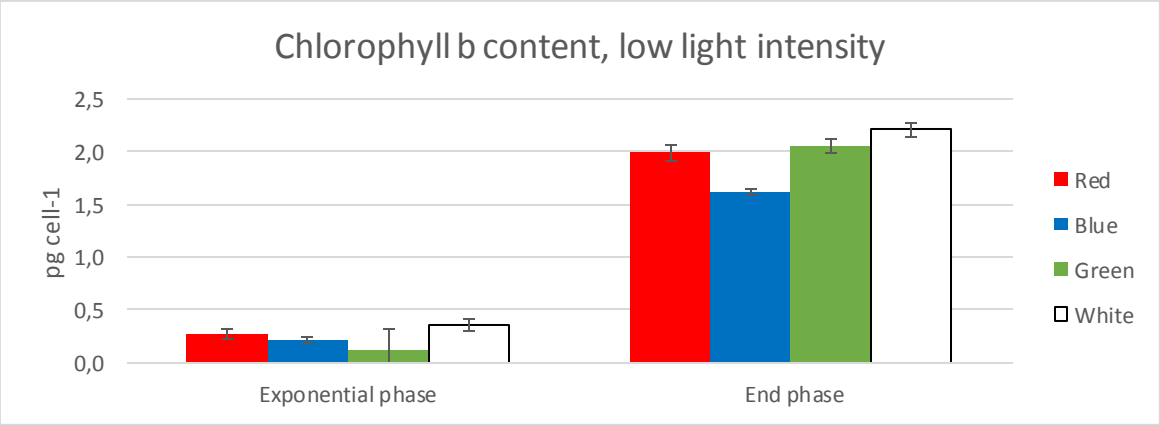


Figure 31: *Chlorophyll b content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under low light conditions (8 ± 10 µmol m^2 sec^{-1}).*

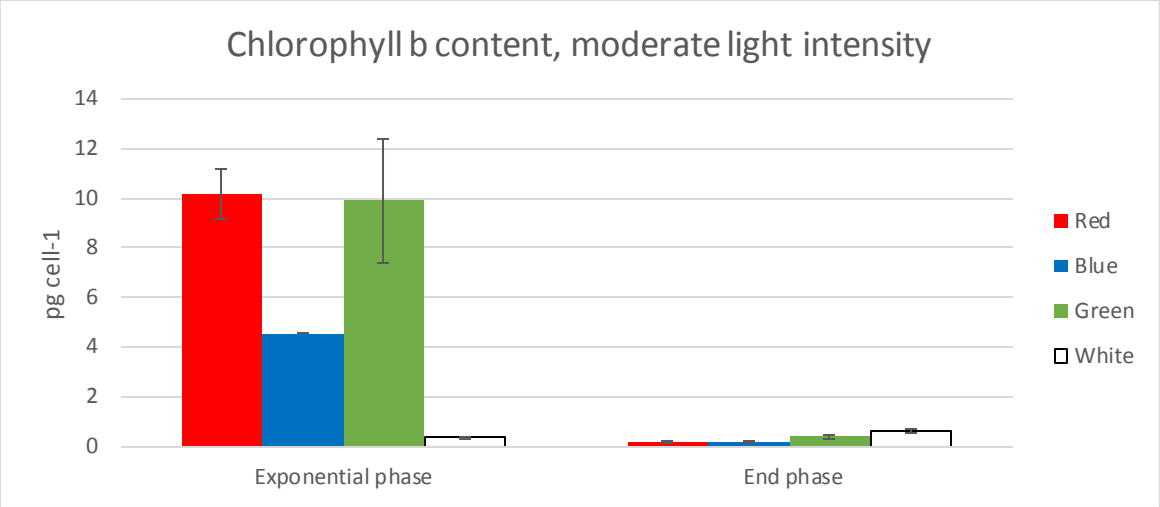


Figure 32: *Chlorophyll b content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

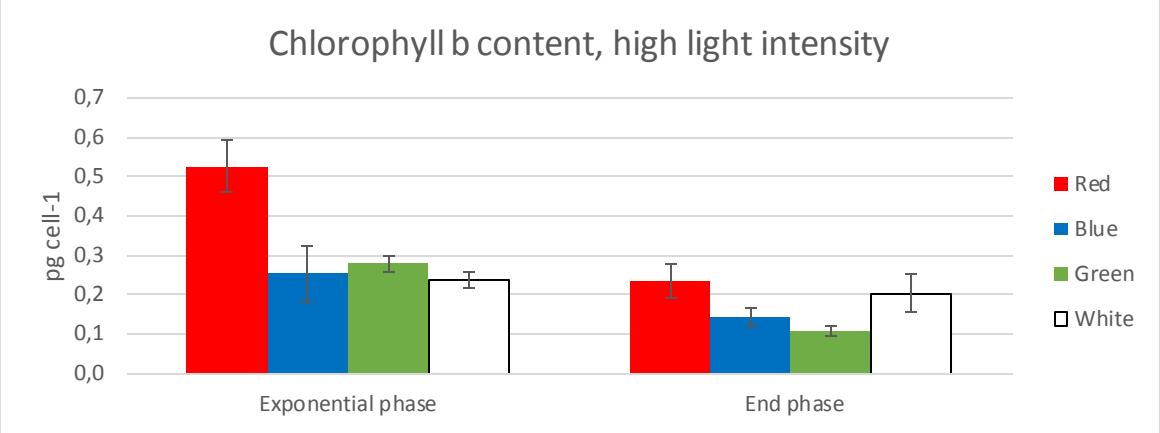


Figure 33: *Chlorophyll b content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 20 µmol m^2 sec^{-1}).*

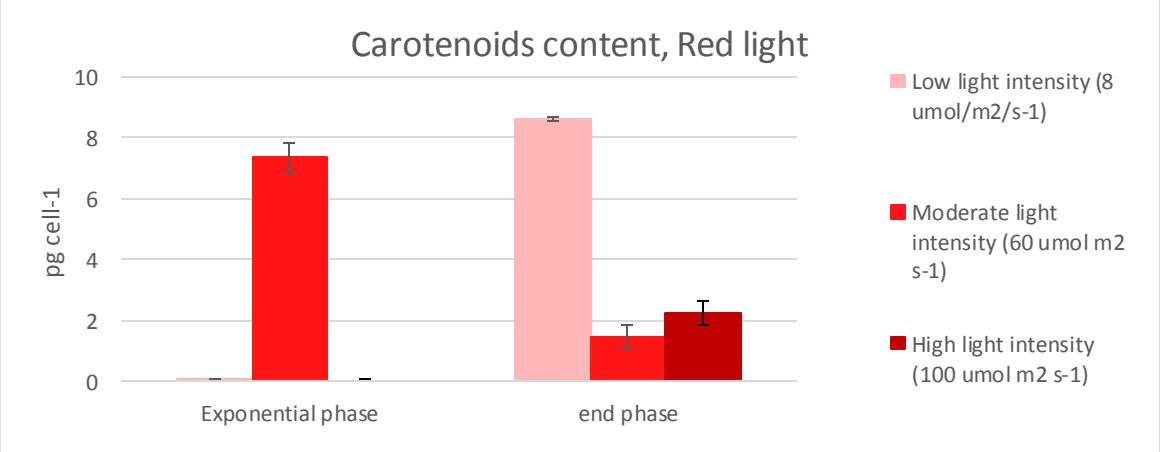


Figure 34: *Carotenoid content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under low light, medium light and high light conditions.*

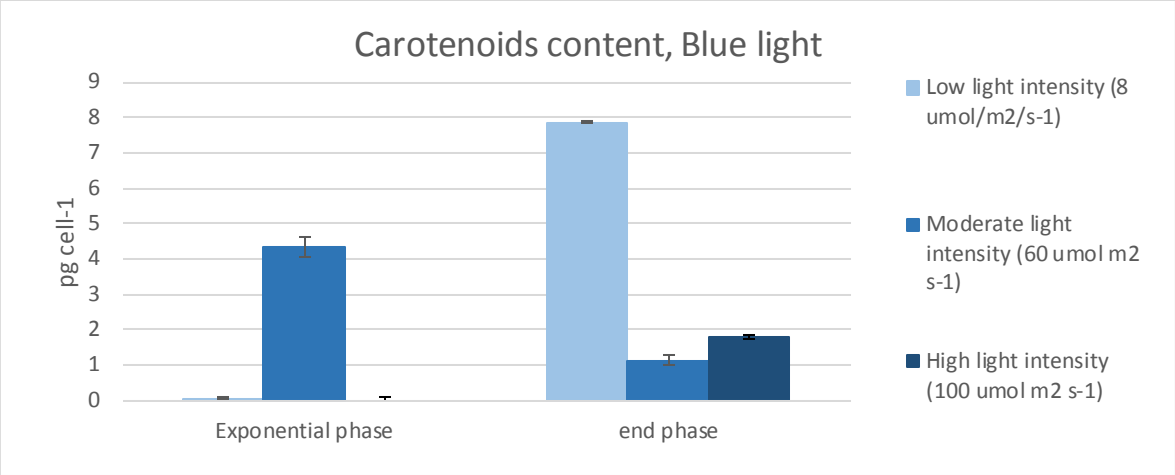


Figure 35: *Carotenoid content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under low light, medium light and high light conditions.*

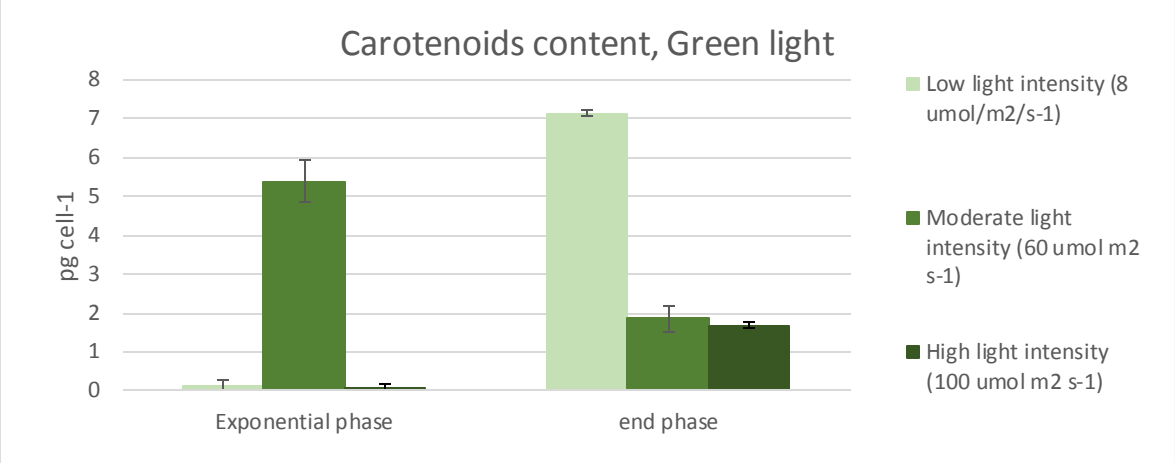


Figure 36: *Carotenoid content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under low light, medium light and high light conditions.*

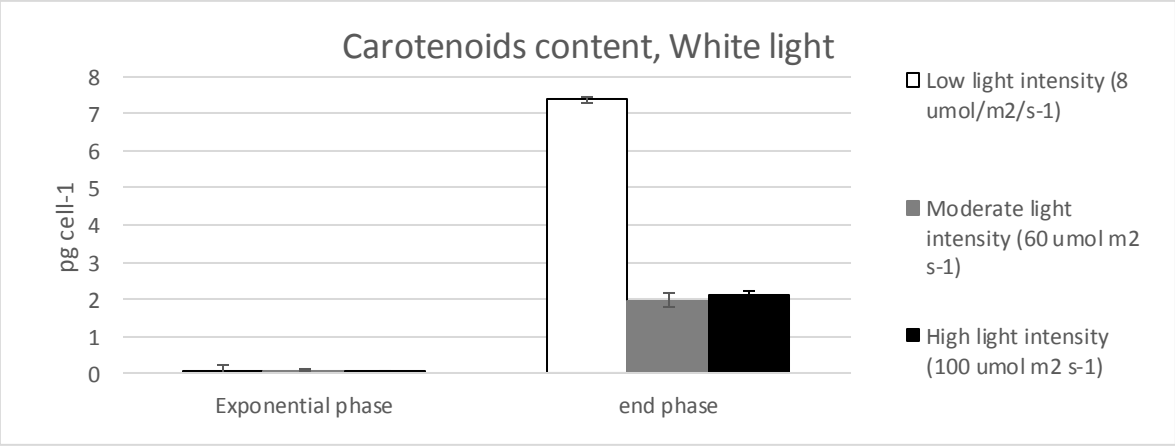


Figure 37: *Carotenoid content in Rhodomonas sp. cultures exposed to white light (λ_{peak}: 415 - 720 nm) under low light, medium light and high light conditions.*

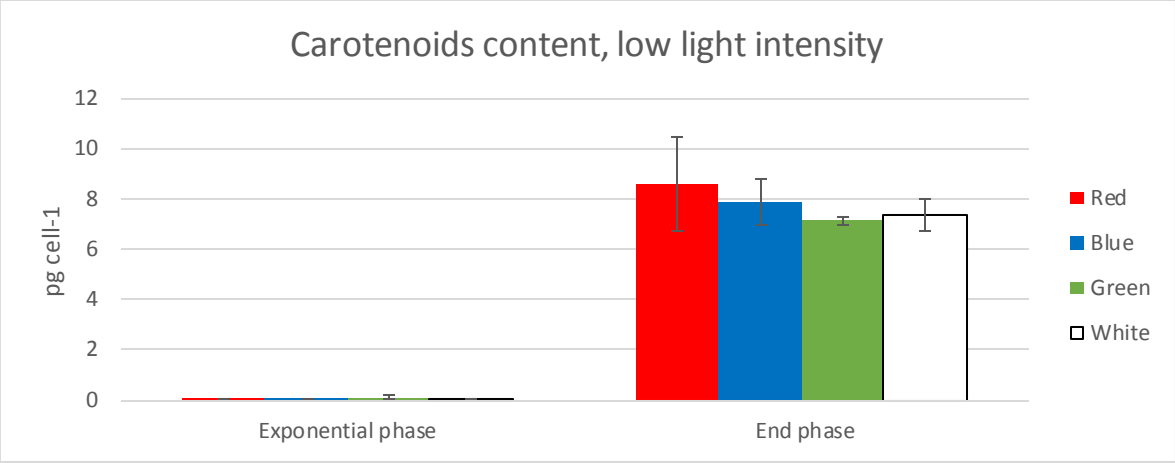


Figure 38: *Carotenoid content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under low light conditions (8 ± 10 µmol m^2 sec^{-1}).*

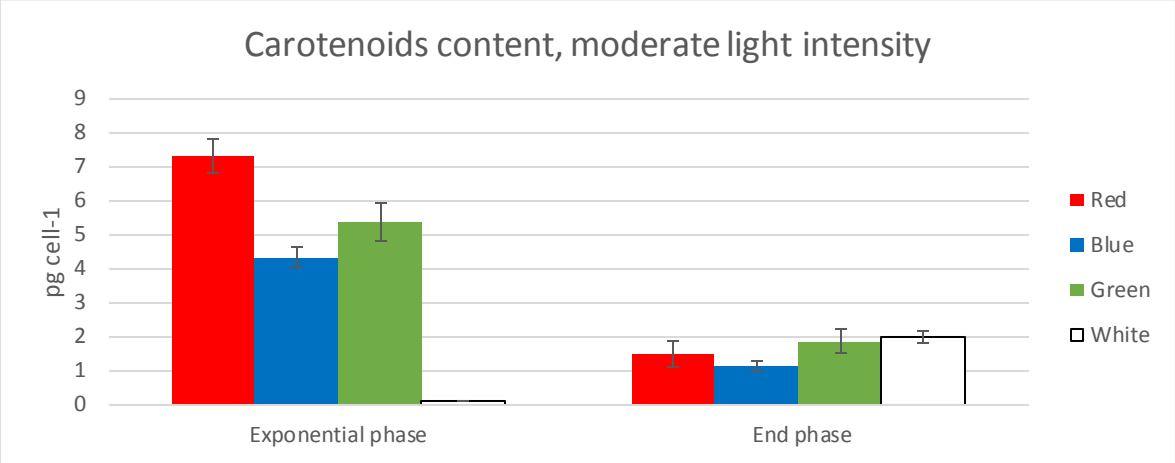


Figure 39: *Carotenoid content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

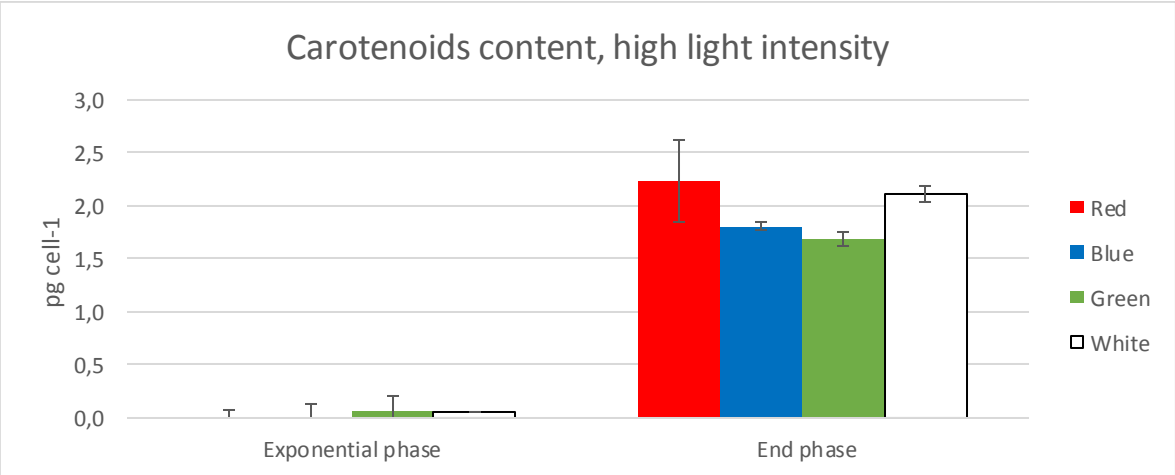


Figure 40: *Carotenoid content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 10 µmol m^2 sec^{-1}).*

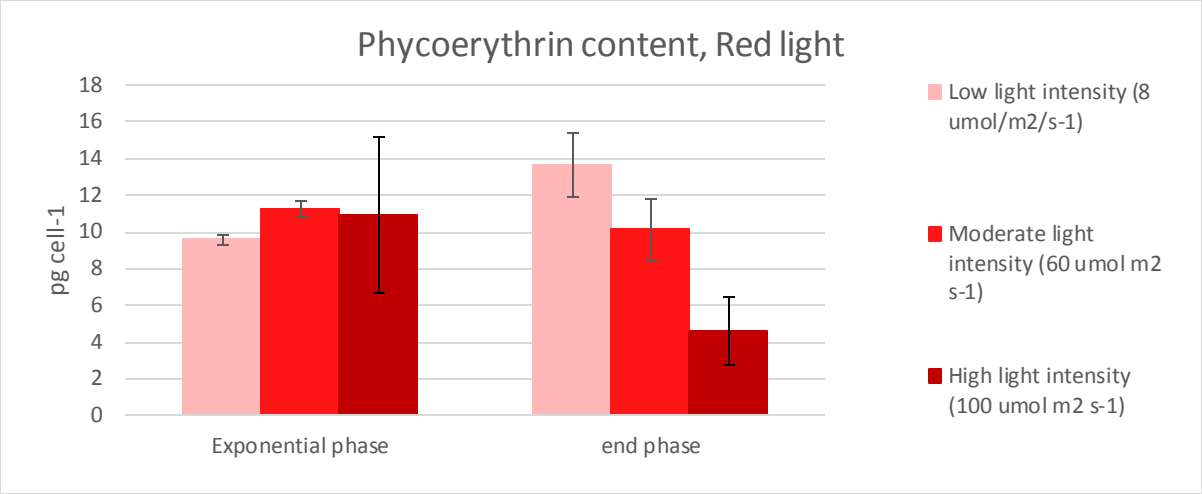


Figure 41: *Phycoerythrin content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under low light, medium light and high light conditions.*

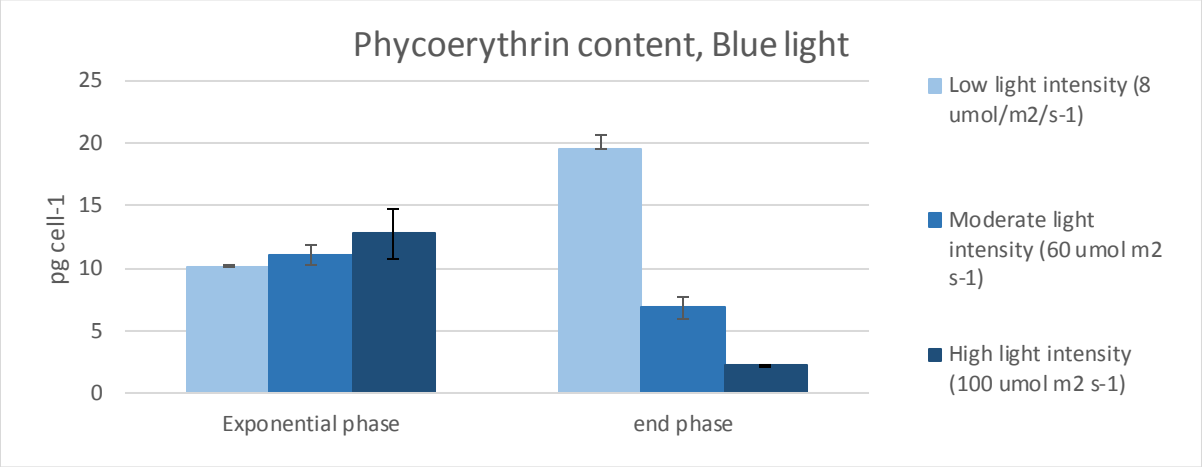


Figure 42: *Phycoerythrin content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under low light, medium light and high light conditions.*

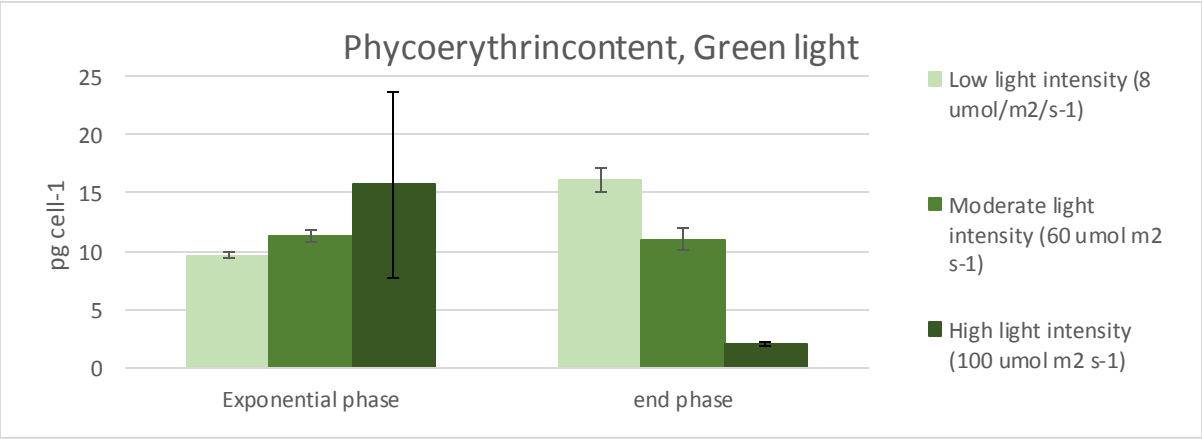


Figure 43: *Phycoerythrin content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under low light, medium light and high light conditions.*

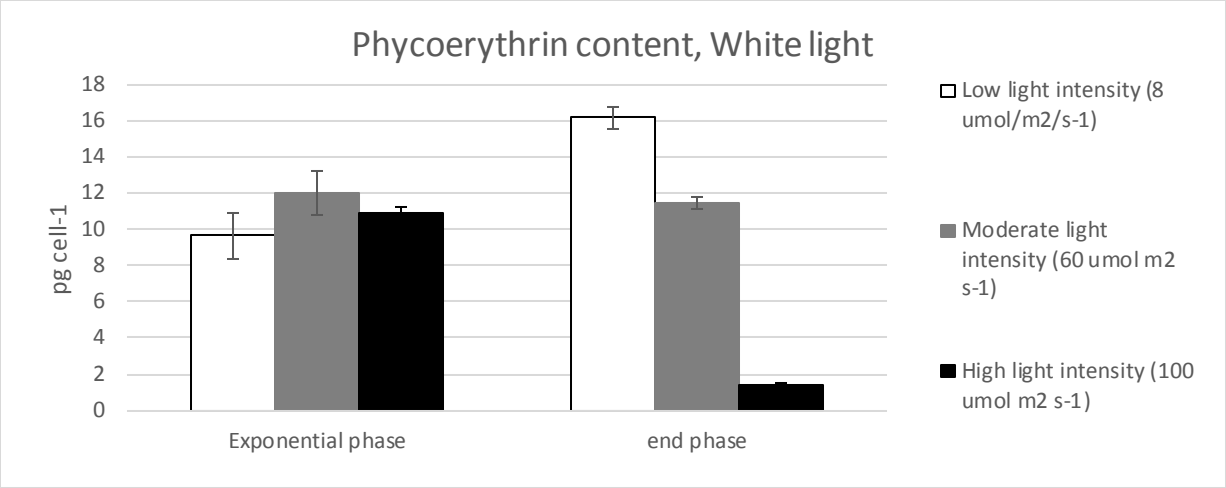


Figure 44: *Phycoerythrin content in Rhodomonas sp. cultures exposed to white light (λ_{range}: 415 - 720 nm) under low light, medium light and high light conditions.*

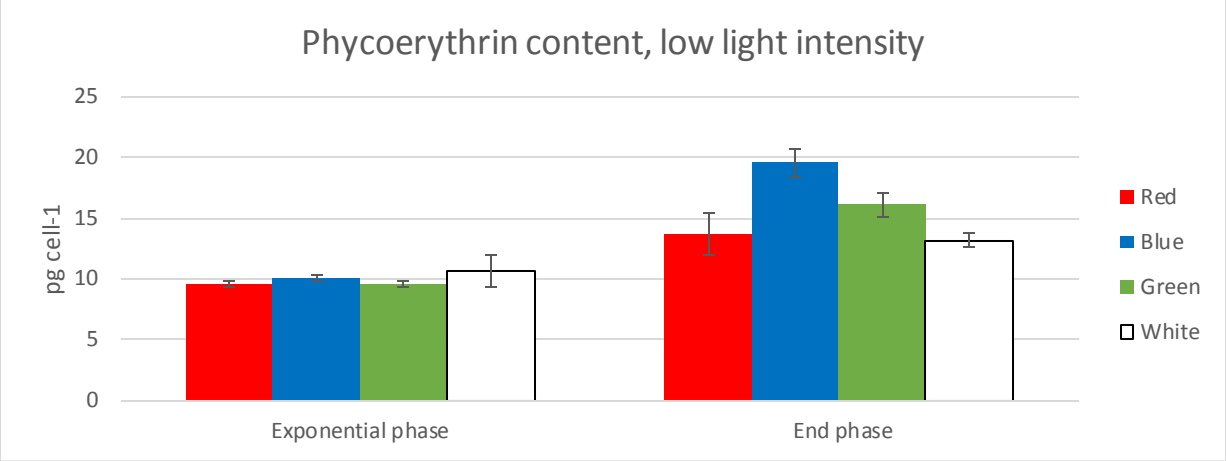


Figure 45: *Phycoerythrin content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under low light conditions (8 ± 10 µmol m^2 sec^{-1}).*

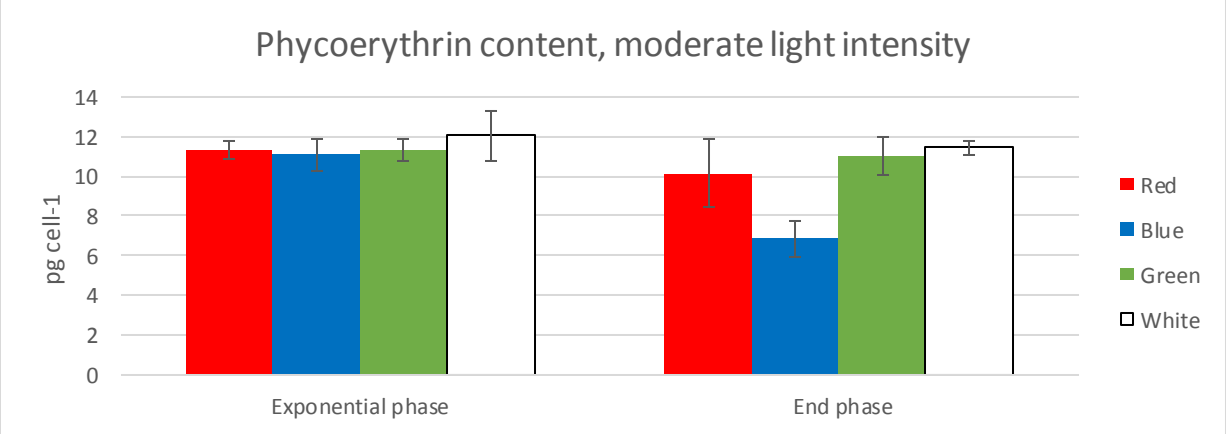


Figure 46: *Phycoerythrin content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

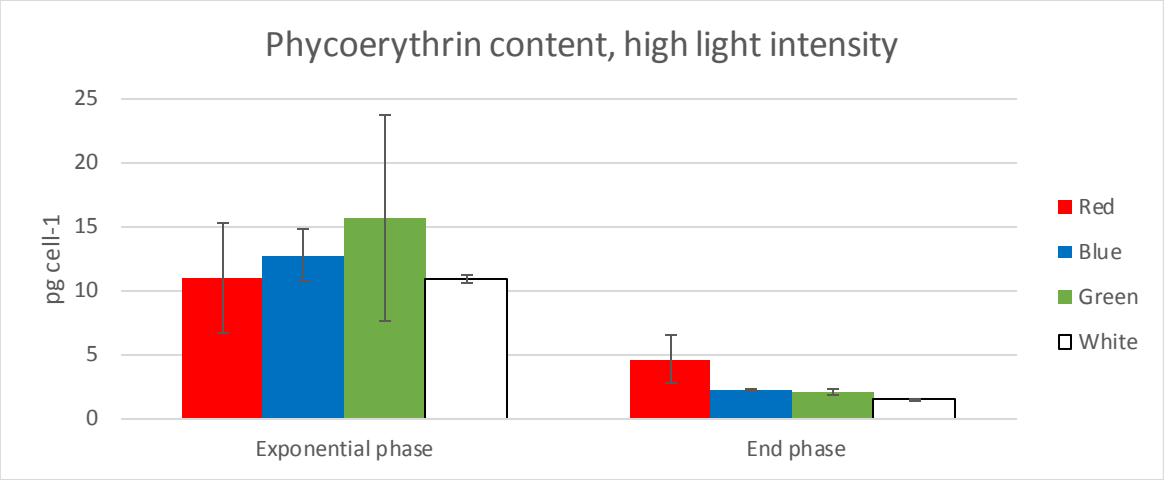


Figure 47: *Phycoerythrin content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 20 µmol m^2 sec^{-1}).*

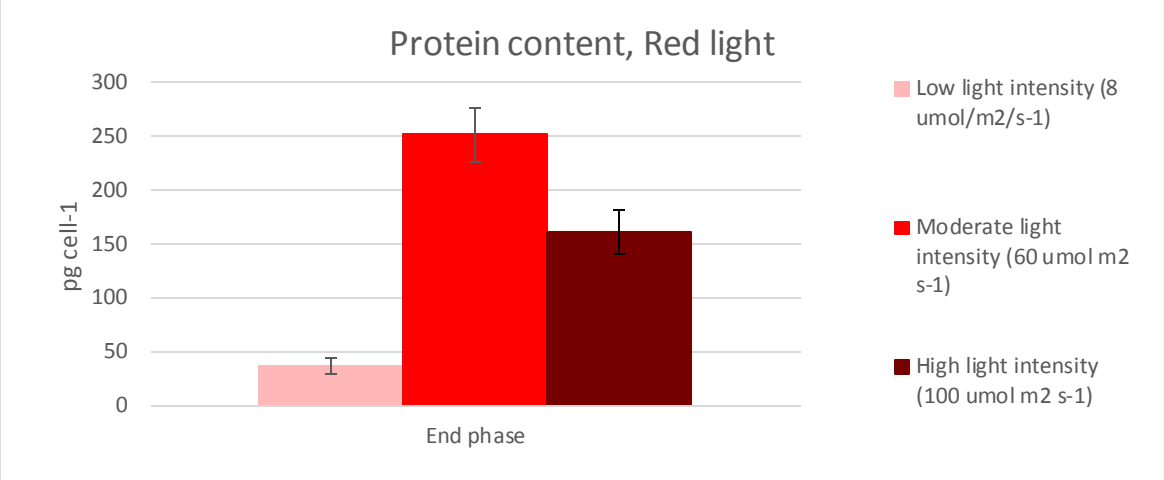


Figure 48: *Protein content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under low light, medium light and high light conditions.*

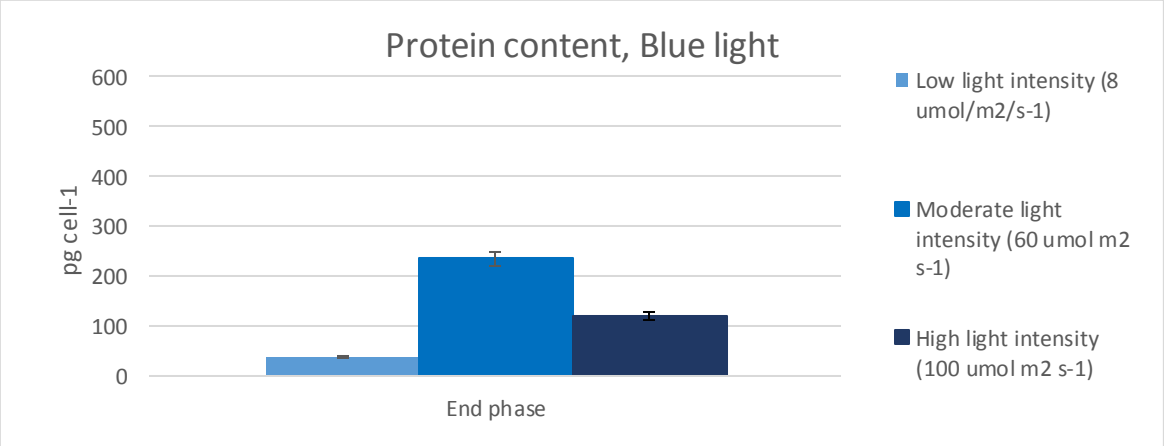


Figure 49: *Protein content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under low light, medium light and high light conditions.*

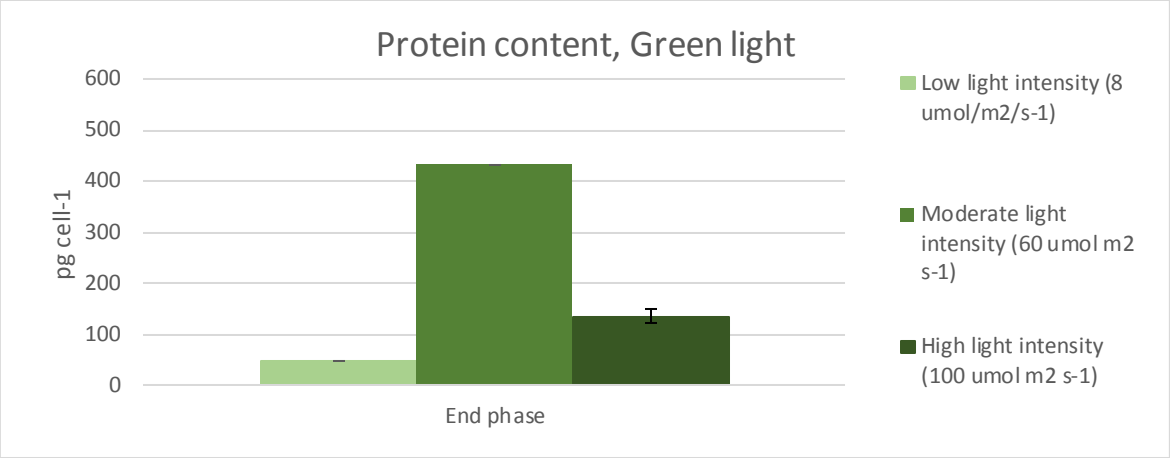


Figure 50: *Protein content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under low light, medium light and high light conditions.*

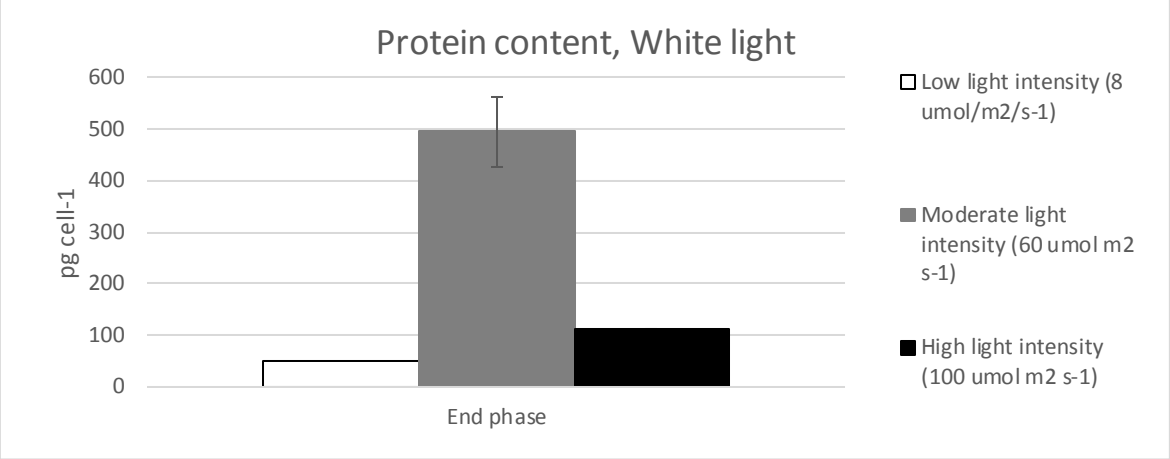


Figure 51: *Protein content in Rhodomonas sp. cultures exposed to white light (λ_{range}: 415 - 720 nm) under low light, medium light and high light conditions.*

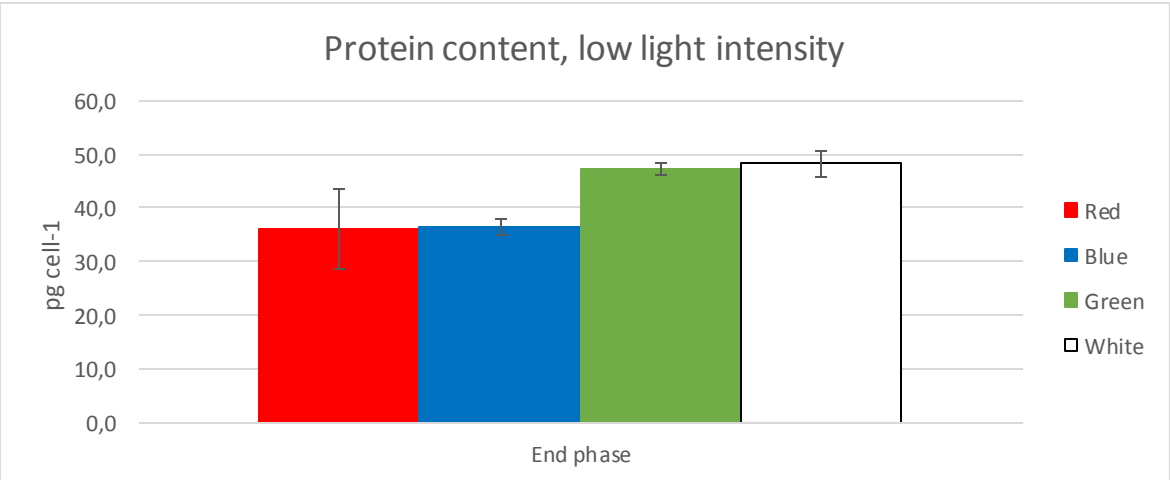


Figure 52: *Protein content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under low light conditions (8 ± 10 µmol m^2 sec^{-1}).*

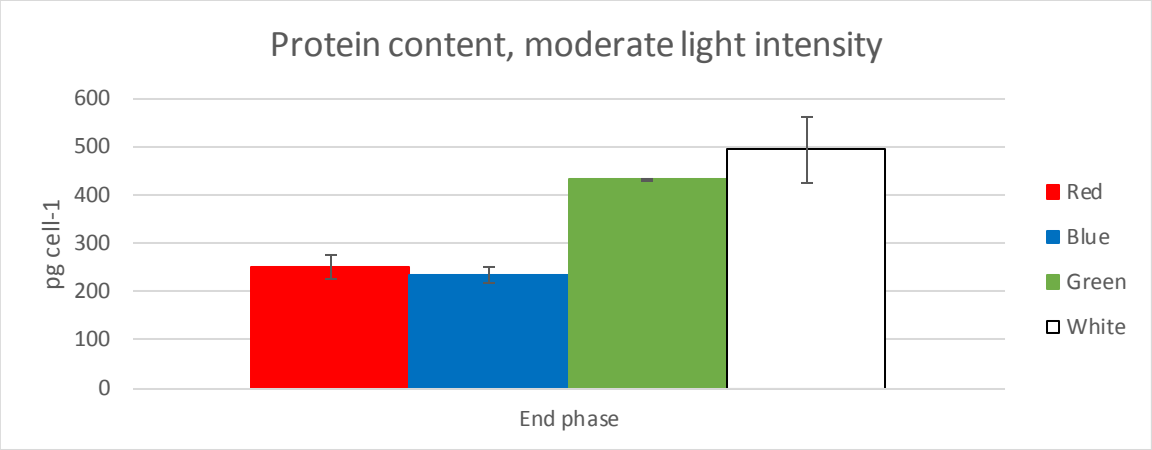


Figure 53: *Protein content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

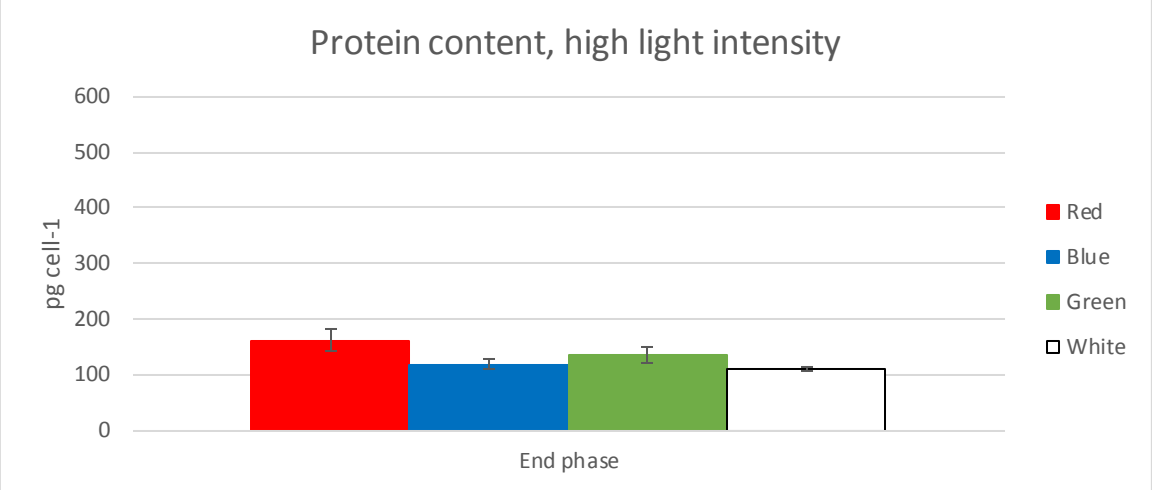


Figure 54: *Protein content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 20 µmol m^2 sec^{-1}).*

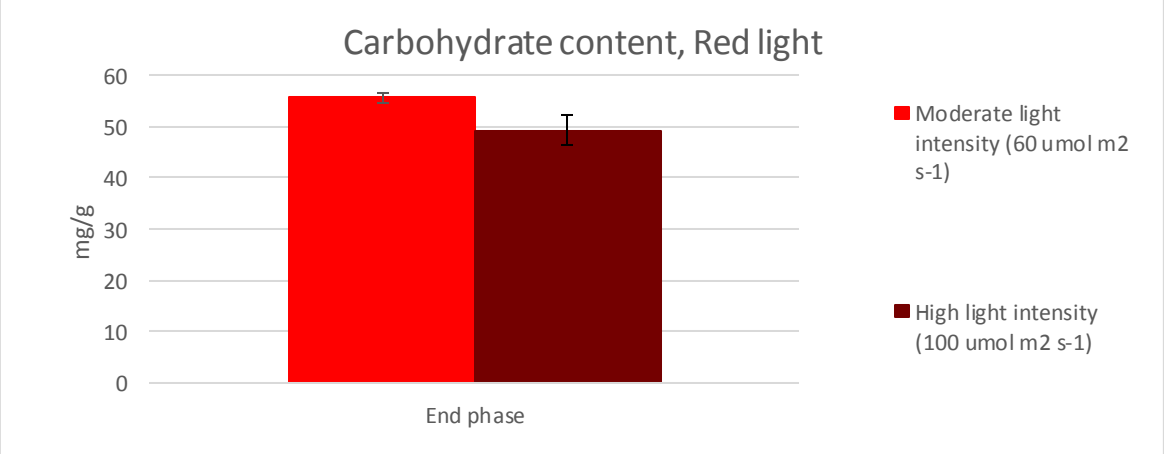


Figure 55: *Carbohydrate content in Rhodomonas sp. cultures exposed to red light (λ_{peak}: 630 nm) under medium light and high light conditions.*

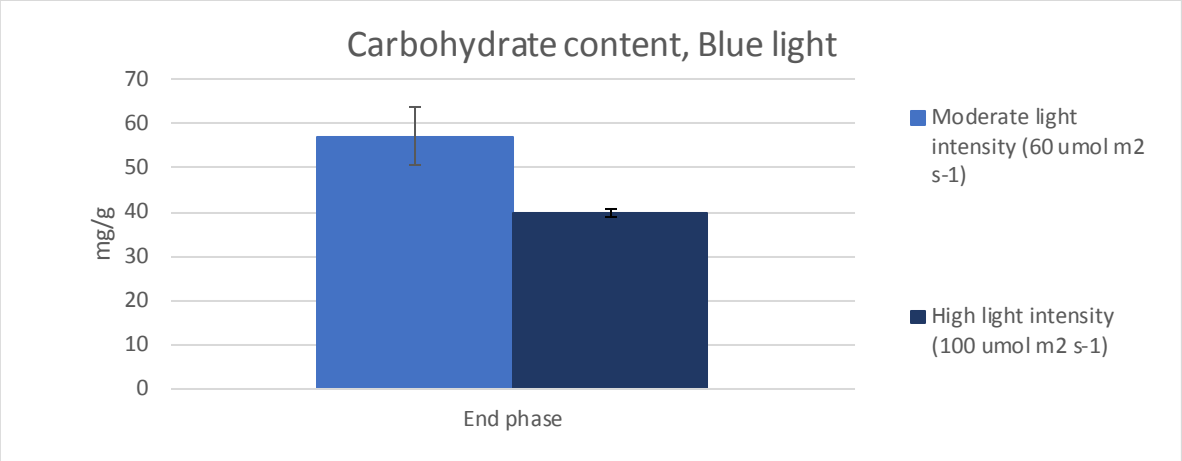


Figure 56: *Carbohydrate content in Rhodomonas sp. cultures exposed to blue light (λ_{peak}: 461 nm) under medium light and high light conditions.*

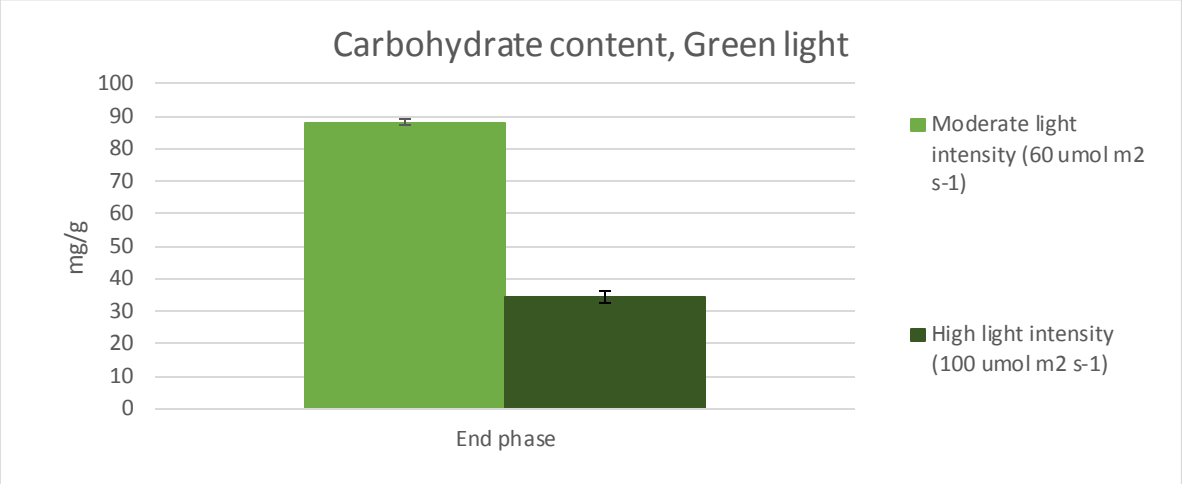


Figure 57: *Carbohydrate content in Rhodomonas sp. cultures exposed to green light (λ_{peak}: 517 nm) under medium light and high light conditions.*

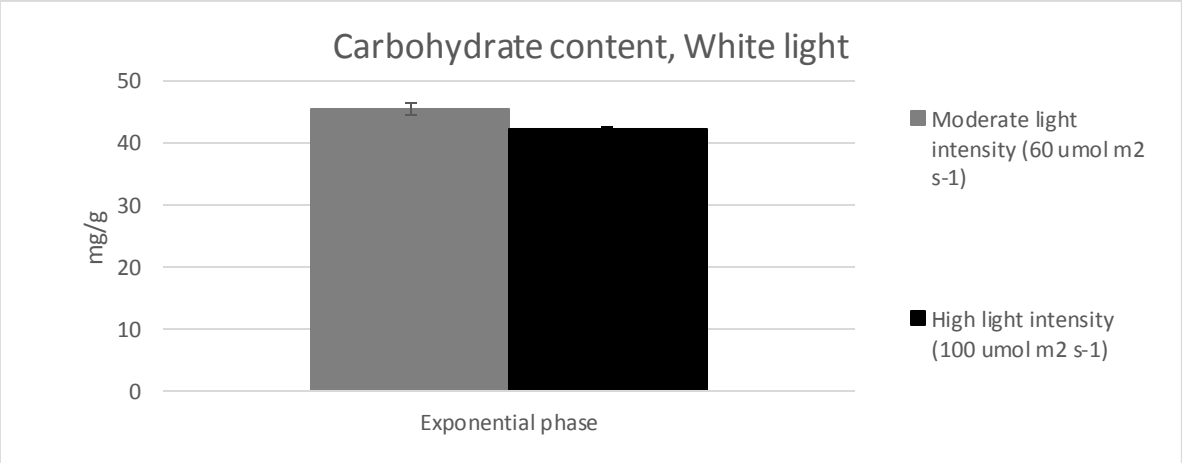


Figure 58: *Carbohydrate content in Rhodomonas sp. cultures exposed to white light (λ_{range}: 415 - 720 nm) under medium light and high light conditions.*

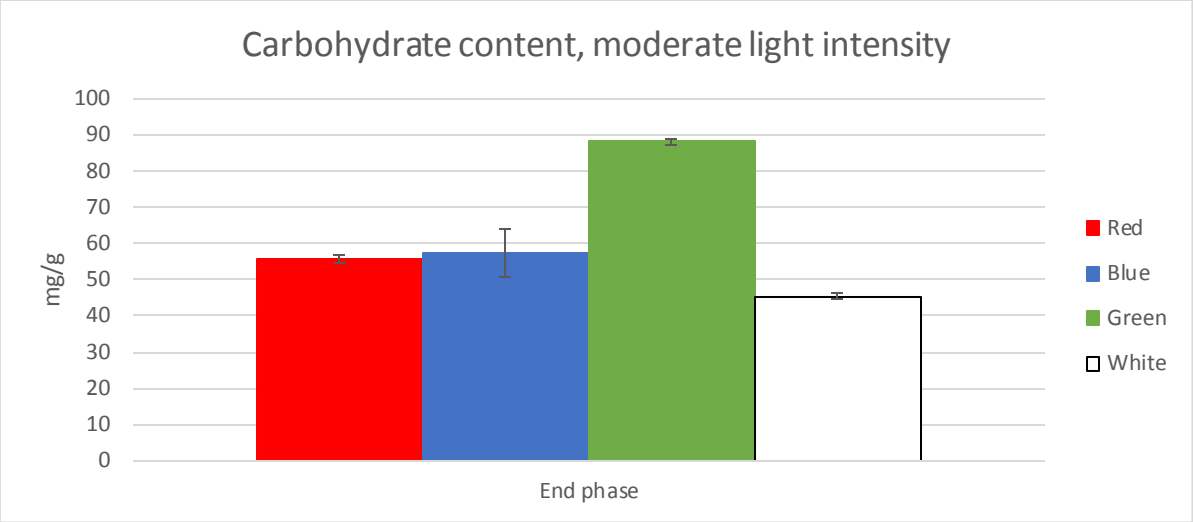


Figure 59: *Carbohydrate content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under medium light conditions (60 ± 10 µmol m^2 sec^{-1}).*

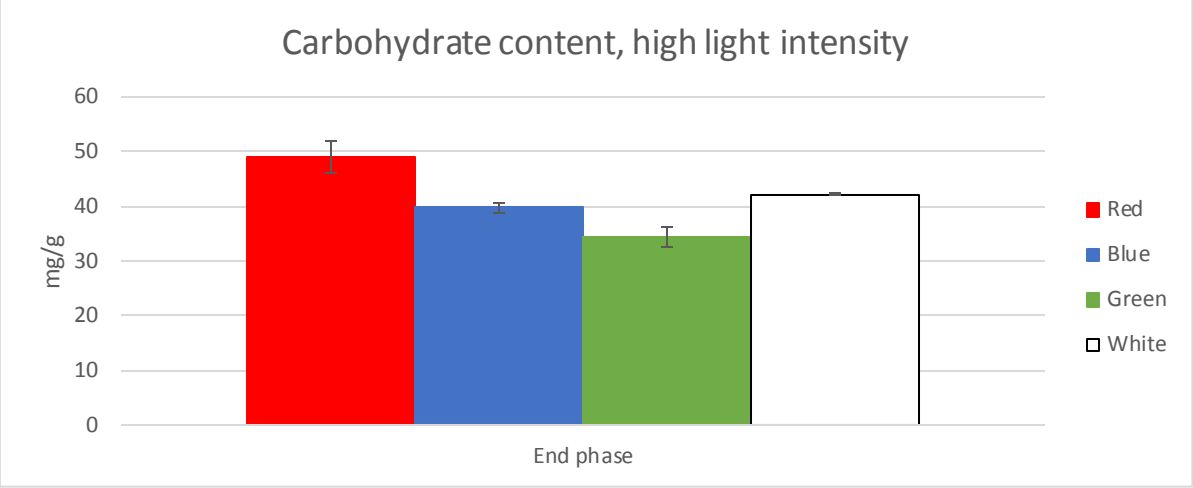


Figure 60: *Carbohydrate content in Rhodomonas sp. cultures exposed to wavelength colours red, blue, green and white under high light conditions (80 ± 20 µmol m^2 sec^{-1}).*