The effect of light colour on the volumetric productivity and maximum biomass yield on light energy of *Chlamydomonas reinhardtii*

Christos Latsos

Wageningen UR

August 2014

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MSc Thesis

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Author: Christos Latsos
Registration number: 891003504040
Supervisors: Tim de Mooij, Msc
Dr. Ir. Marcel Janssen
Examiner: Dr. Ir. Mathieu Streefland
Course code: BPE-80336

Duration: February 2014 – August 2014

Chair group Bioprocess Engineering

Contents

Summary

Microalgae convert light energy to chemical energy through the process of photosynthesis, which they use to produce biomass. Despite light is the driving force of photosynthesis, excessive amounts of light cause over-saturation of the photosynthetic electron transport chain and eventually result in the inactivation of the photosynthetic machinery. During oversaturation on the one hand algae grow in their maximal growth rate, but on the other hand the light converting efficiency to biomass is low, because a fraction of incoming light is dissipated as heat and fluorescence. In order to minimize and prevent the light waste, algae mutants with truncated antenna complexes can be used. Another option that supports the first one is the use of a light source which is not strongly absorbed. This report investigated the hypothesis that a lower light absorption rate per cell results in a higher cell concentration and a higher volumetric productivity, while higher light absorption rate per cell will lead to oversaturation and heat dissipation, lower cell concentration and lower volumetric productivity. Microalgae don't absorb with the same efficiency in all light wavelengths. *Chlamydomonas reinhardtii* was cultivated under different light colours (white, blue, deep red, orange red, amber and amber-blue) in order to determine the volumetric productivity and the maximum biomass yield on light. Different colours were used to achieve different light absorption rate per cell. The cultivation was continuous and the mass culture conditions was simulated with high biomass and high light intensity. For the volumetric productivity microalgae were cultivated in turbidostat mode and chemostat mode to ensure a constant light regime. A mathematical model was used in order to predict the results of these experiments. According to the model the highest volumetric productivity was predicted for amber light and it was equal with 4.5 g $L^{-1}d^{-1}$, while the lowest was predicted for blue light, 1.7 g $L^{-1}d^{-1}$.

The volumetric productivity for amber light was lower compared to the predicted one, thus a small amount of blue light (50 µmol m^{-2} s^{-1}) was added in order to fully activate the photosystem II of the organism. This decision was taken because cultivation under blue light showed better photosystem II function. When the cultures were in steady state, i.e. five consecutive days in which a stable biomass dry weight content and specific growth rate state were obtained, the highest calculated volumetric productivity was for amber-blue light, 3.9 g $L^{-1}d^{-1}$, while the lowest was obtained for deep-red, 1.3 g L^{-1} d^{-1}. The biomass yield on light measurements showed the same differences for each light colour as for volumetric productivity.

The absorptive cross section experimental data supported the theory that blue light is easier absorbed, that's why for blue light it was lower than for the other colours. Moreover, the Fv/Fm ratio showed that algae cultivated under white and blue light had the highest maximal quantum yield compare to the other colours, 0.64 and 0.63 respectively. That means that during these experiments microalgae under blue or white light were healthier than under other colours.

Experiments to investigate the maximum yield on light were conducted to give a better explanation of the volumetric productivity experiments and make a fair comparison of the productivities of cultures under different light colours. The maximum biomass yield on light was obtained for white and blue light by performing chemostat experiments under low light intensities, 80 µmol·m^{-2}·s^{-1} and 30 µmol m^{-2} s^{-1} respectively. The result was 1.64 g mol^{-1} and 1.48 g mol^{-1} for white and blue light respectively.

To sum up, these results supported the hypothesis that low specific light uptake per cell, such as amber light with the addition of a bit of blue light, results in high volumetric productivity. Moreover, the addition of blue light showed that can trigger and increase the photosynthetic efficiency of the

organism.

Introduction

Microalgae are unicellular microorganisms and are considered as a very promising alternative for a broad range of industrial application such as cosmetics, food, feed and biofuel production (Pires et al. 2012). The main advantage of microalgae is their high productivity compared to conventional crops and their ability to sustainably produce proteins, carotenoids, fatty acids and carbohydrates, and that they can be cultivated at non-arable land (Wijffels 2010; Melis et al. 1999). Moreover microalgae can grow under different conditions, such as autotrophic, heterotrophic, mixotrophic and photoheterotrophic conditions. The only energy source of microalgae is light because they are all photoautotrophs and use light in order to synthesize metabolites and biomass. Therefore, in a microlagal culture light is the most crucial parameter to optimize productivity.

However, algae cultivation is expensive and this cost should be decreased. One limitation to this is the low yield in high light intensity, which can be solved by reducing the light absorption rate. One option is to reduce the pigment composition by creating mutants with truncated antenna systems, which are complexes of proteins and chlorophyll molecules and are able to absorb photons. A smaller antenna results in less light absorption per cell, but more efficient light use per cell, because oversaturation of the photosystems and concomitant dissipation of light energy, are lower (Nakajima et al. 2001). The principle of antenna size reduction is a promising strategy to increase productivity. Tim de Mooij (2014) indicates that despite antenna mutants have lower light absorption capacity than wild type, their productivity was not as much higher than wild type as it was expected.

Although not applicable under outdoor conditions, in the laboratory a low pigment content can be simulated by reducing the amount of absorbed light per cell by adjusting light colour. Algae absorb differently light of different wavelengths. For instance wavelength of white light range between 400 and 700 nm, which wavelengths cannot be absorbed equally by algae. Using monochromatic light, which consists of small range of wavelengths, different absorption rate per cell can be achieved and as a result different rate of photosynthesis. When the absorbance of light is lower there is less saturation and less light is wasted as heat or fluorescence. Moreover in a lower absorption light per cell more algae can be grown at the same amount of light. When algae absorb less light but the ingoing light is constant, more algae can be grown in the reactor. Thus the use of a light colour which is not well absorbed would be essential for the increase of volumetric productivity, because the conversion of light to biomass would be more efficient. In other words, when a culture is illuminated by a monochromatic light hardly absorbed, the algae will show lower specific growth rate because the photosynthesis rate will be lower, but the cell density in the reactor will be higher. According to the literature red light (660±20nm) is the optimal light wavelength for microalgae cultivation, because red light can be more efficiently absorbed by the chlorophylls (Matthijs et al. 1996; Baba et al. 2012; Kim et al. 2014). However another theory is that green light penetrates deeper in a culture so this can lead to more efficient photosynthesis (Terashima et al. 2009). The second one refers to plants (sunflowers) but it could work in algae as well.

In this thesis report we will try to investigate the correlation between light absorption per cell and the accompanied productivity. *Chlamydomonas reinhardtii* will be cultivated continuously under different light colours at high light intensities in order to calculate the volumetric productivity. The intensities will be high because in these intensities not light absorption, but light use efficiency is the limiting factor. Moreover, to support and give a better explanation of the results of the volumetric productivity

experiments, maximum yield on light will be measured. The g of biomass that can be produced per mol of photon will be measured, which is maximal in low light intensities, because the antenna complexes in photosystem are not saturated. These experiments will investigate what the maximal efficiency is that can be reached when the algae are exposed to different light colours and make a fair comparison of the productivities. For instance, if there is a significant difference in maximum yield on light between the different colours, it may have influenced the productivity values that were measured during the volumetric productivity experiments. The maximal yield of biomass was measured in a chemostat mode under blue (460±50nm) and white light at low light intensities, where the algae work at maximal efficiency because there is no light saturation at these intensities.

Furthermore, we will try to investigate photosystem II (PSII) efficiency using fluoremetry. Direct reactor samples were taken every day to measure the Fv/Fm ratio using the Aquapen device and for absorptive cross section area using a spectrophotometer with an integrating sphere. The absorbed light follows three pathways: used in ATP and NADPH production, re-emitted as fluorescence or dissipated as heat. The Fv/Fm ratio compares the dark-adapted microalgae pre-photosynthetic fluorescent state, called minimum fluorescence to maximum fluorescence, called Fm.

A productivity model for this algae will be used by which the theoretical biomass concentration and the productivity can be calculated. This productivity model is a useful tool to determine what wavelength of light will give the highest productivity.

It is expected that monochromatic light which is not so strongly absorbed will lead in lower specific growth rate, because the photosynthetic rate will be lower. However when the absorption per cell is lower but the amount of light constant more algae can be grown in the reactor, therefore the biomass yield on light will be higher. Hence in hardly absorbed light algae have lower specific growth rate but higher maximal cell density. More specifically we expect that amber light (600±75nm) will result in higher volumetric productivity, because it is hardly absorbed compare to the other colours. On the other hand blue and red light, which are better absorbed per cell, will result in higher specific growth rate but not efficient light conversation to biomass.

If the hypothesis and our expected results become true, it will be a great investigation for the microalgae file. The results will support the promising technology of antenna mutants, because if amber light results in higher volumetric productivity, mutants with smaller antenna complexes will have the same increased productivity.

Aim

The aim of this project is to show that less light absorption rate per cell leads to higher volumetric productivity and biomass yield on light, which will be calculated by cultivating the alga *Chlamydomonas reinhardtii* under different light colours (white, blue, deep red, orange red, amber). Monochromatic light that is hardly absorbed by algae will help to achieve the low light absorption rate per cell conditions. This report is a fundamental research, which will support the theory that genetically engineered algae with reduced pigments can work. This research is not applicable outside and on a large scale because it is not cost effective to apply coloured light on scale up reactors.

Theory

Microalgae

Microalgae are small (1-50 micrometers diameter) prokaryotic (Cyanobacteria) or eukaryotic organisms and they can be found both in fresh and salt water. Despite the many thousands of microalgae species, only some thousands of them have been described. Most of them contain chlorophyll in order to function as sunlight driven cell factories. During their photoautotrophic growth they convert inorganic carbon into biomass. Moreover, microalgae have become a promising alternative feedstock for food and biochemical products, which led to many research areas in this field. (Olaizola 2003; Harun et al. 2010; Pulz & Gross 2004)

Applications of microalgae

Microalgae have a big variety of applications. First of all, algae are useful for nutritional purposes and can be found in snack foods, beverages and candy bars. Algae are able to provide a huge variety of vitamins, something that makes them useful in nutrition. (Yamaguchi 1996)

Moreover many microalgae have beneficial effects in cosmetics, such as in face or anti-aging creams, hair care products and sun protectors. Another application is in animal feed because they have high amounts of proteins and fatty acids. For instance, microalgae produce pigments such as astraxanthin and β-carotene, which are used as fish feed. (Lorenz & Cysewski 2000)

Except for applications in animal feed, microalgae contain fatty acids that can be beneficial for human health. Fatty acids from fish oil cannot cover the human needs, so other sources of fatty acids have to be investigated. (Nettleton 1993)

Last but not least, microalgae can have a high oil content and a high biomass productivity, which makes them potentially useful source for biofuel production. Recently, the use of microalgae as alternative biodiesel feedstock is considered to be of high interest by researchers and the general public. (Priyadarshani & Rath 2012)

Chlamydomonas reinhardtii

Chlamydomonas reinhardtii (Figure 1) is a green microalgae, unicellular (around 10μm diameter), and it can be found in freshwater. It consists of a chloroplast and an "eye" which perceives light, and these are enclosed in the cell wall rich in hydroxyproline rich glycoproteins. Moreover, it can move thanks to its two anterior flagella. It grows on a medium rich in inorganic salts during day using photosynthesis, but it can also grow in the absence of light if there is any alternative carbon source such are organic molecules. (Falkowski & Raven 1997)

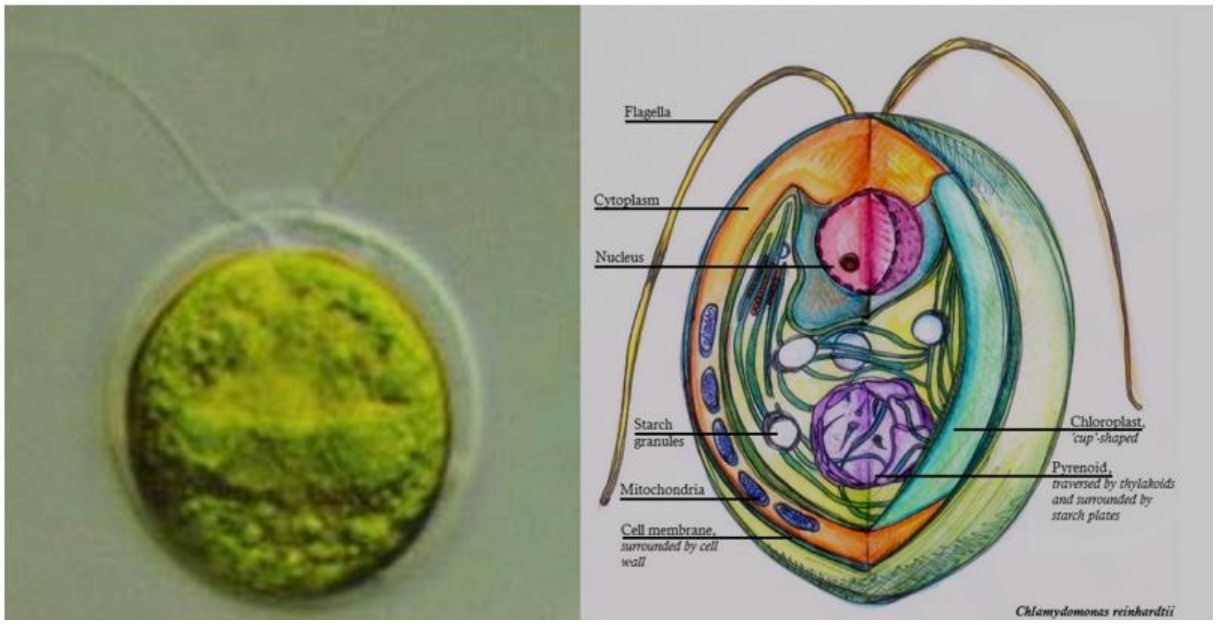

Figure 1: Chlamydomonas reinhardtii: On the left side a microscopic picture of the microorganism is depicted and on the right side a schematic overview is illustrated.(Ninghui Shi 2013)

Photosynthesis

Microalgae use light energy as fuel for their metabolism. Photosynthesis is the process by which plants and microorganism convert light energy to chemical energy. This chemical energy is stored as carbohydrate molecules, which are produced by carbon fixation accompanied by water splitting. The result of this reaction is oxygen production. (Equation 1)

$$CO_2(g) + H_2O(l) \xrightarrow{light\ energy} CH_2O(s) + O_2(g) \quad (1)$$

This reaction illustrates that microalgae can convert carbon dioxide (CO_2) and water (H_2O) into organic products (CH_2O) and produce oxygen (O_2).

Photosynthesis occurs in two stages. In the first stage, "light-dependent reactions" or light reactions capture the energy of light and use it to make the energy-storage molecules ATP and NADPH. During the second stage, the light-independent reactions use these products to capture and reduce carbon dioxide. Equation 1 can be modified to the next two reactions:

$$2H_2O + Light \xrightarrow{Ch1\ a} 4H^+ + 4e + O_2 \quad (2)$$

Where *Chl α* is the plant pigment chlorophyll α. This equation represents the "light reactions" of photosynthesis and it is an oxidation process, where electrons are extracted from water to form molecular oxygen. The other reaction, the reduction of CO_2 can be described by equation (3):

$$CO_2 + 4H^+ + 4e^- \rightarrow CH_2O + H_2O \quad (3)$$

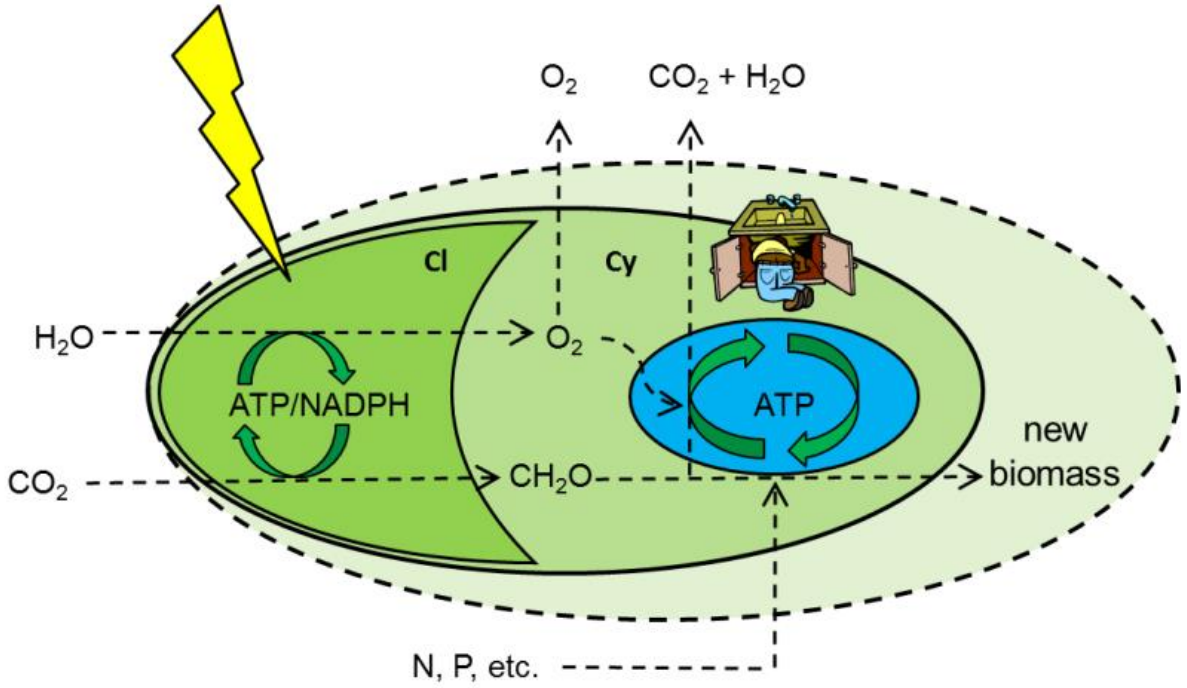

Figure 2: Schematic overview of the complete photoautotrophic growth process of microalgae (Janssen & Lamers 2013)

The light reaction happens in the thylakoid membrane and converts light energy to chemical energy. This chemical reaction must, therefore, take place in the light. Chlorophyll and several other pigments such as beta-carotene are organized in clusters in the thylakoid membrane and are involved in the light reaction. Each of these differently-colored pigments can absorb a slightly different color of light and pass its energy to the central chlorphyll molecule to do photosynthesis.

The first step in light reactions is the light absorption in photosystem II in the thylakoid membranes (Figure 3). The energy of light, which is captured by P680 reaction center, activates electrons to leave the chlorophyll molecules in the reaction center. These electrons pass through a series of cytochromes in the nearby electron-transport system in order to enter the photosystem I. In the next step, the spent electrons from P680 enter the P700 reaction center in photosystem I. Light now activates the electrons, which receive a second boost out of the chlorophyll molecules. There they reach a high energy level. Now the electrons progress through a second electron transport system in order to reduce NADP. This reduction occurs as two electrons join NADP and energize the molecule. Because NADP acquires two negatively charged electrons, it attracts two positively charged protons to balance the charges. As a result, the NADP molecule is reduced to NADPH, a molecule that contains much energy.
Because electrons have flowed out of the P680 reaction center, the chlorophyll molecules are left without a certain number of electrons. Electrons secured from water molecules replace these electrons. Each split water molecule releases two electrons that enter the chlorophyll molecules to replace those that were lost. The third product of the disrupted water molecules is oxygen. Two oxygen atoms combine with one another to form molecular oxygen, which is given off as the byproduct of photosynthesis.
What has been described above are the *noncyclic energy-fixing reactions*. Certain plants are also known to participate in *cyclic energy-fixing reactions*. These reactions involve only photosystem I and P700 reaction center. Excited electrons leave the reaction center, pass through coenzymes of the electron transport system, and then follow a special pathway back to P700. Each electron powers the

proton pump and encourages the transport of a proton across the thylakoid membrane. This process enriches the proton gradient and eventually leads to the generation of ATP. ATP production in the energy-fixing reactions of photosynthesis occurs by the process of chemiosmosis. Essentially, this process consists of a rush of protons across a membrane (the thylakoid membrane, in this case), accompanied by the synthesis of ATP molecules. Biochemists have calculated that the proton concentration on one side of the thylakoid is 10,000 times than on the opposite side of the membrane. (Allen et al. 2012) (Falkowski & Raven 2013)

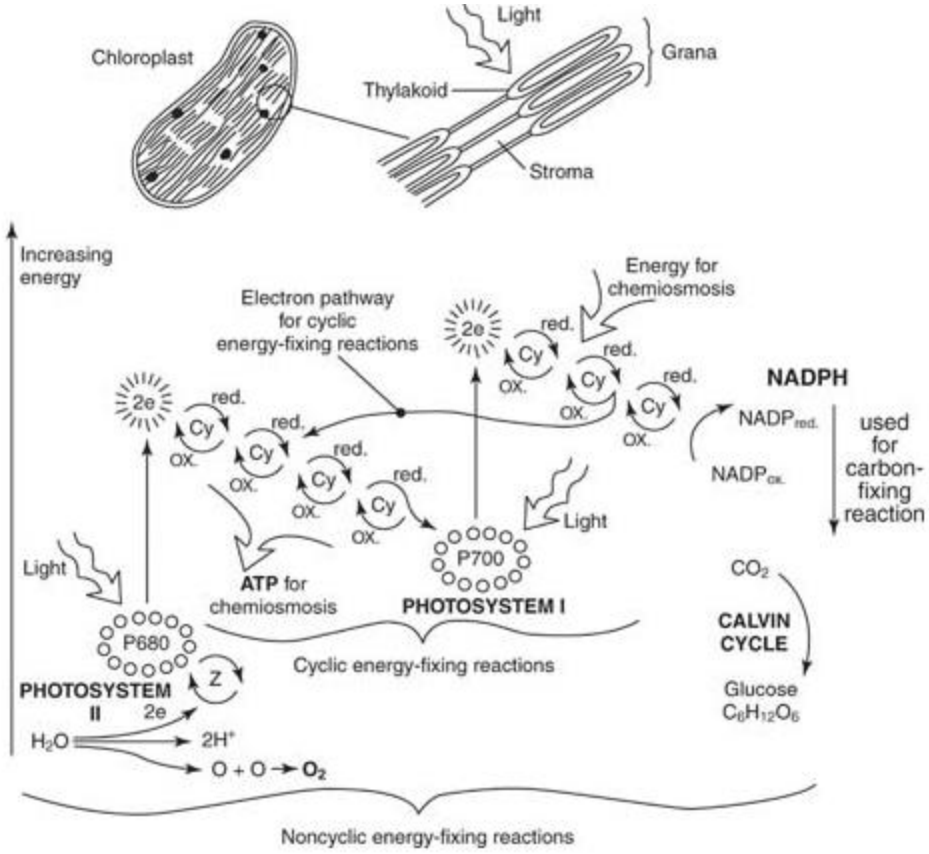

Figure 3: Energy fixing reactions. (Source: Wikipedia. Image by Unknown)

These reactions are of great importance because the ATP and NADPH, which are produced, are used in the Calvin Cycle (Falkowski & Raven 2013). This cycle produces triose, which is converted to algal biomass. Equation 4 presents the overall photoautotrophic growth process.

$$a\,CO_2(g) + b\,H_2O(l) + c\,NH_4{}^+(aq) \xrightarrow{\substack{light\\energy}} d\,CH_{x_h}O_{x_o}N_{x_n}(s) + e\,O_2(g) + f\,H^+(aq) \quad (4)$$

Plants and algae use light-harvesting complexes to collect more light than would be absorbed only by the photosynthetic reaction center. This complexes are part of the photosystem and have been found in a variety of photosynthetic species. They surround the photosynthetic reaction center and consist of proteins and photosynthetic pigments. A photon is absorbed by one of the pigment molecules and transfers that energy by successive fluorescence events to neighboring molecules until it reaches the action center where the energy is used to transfer an energetic electron to an electron acceptor. (Karp G., 2008) (Moore et al, 1995)

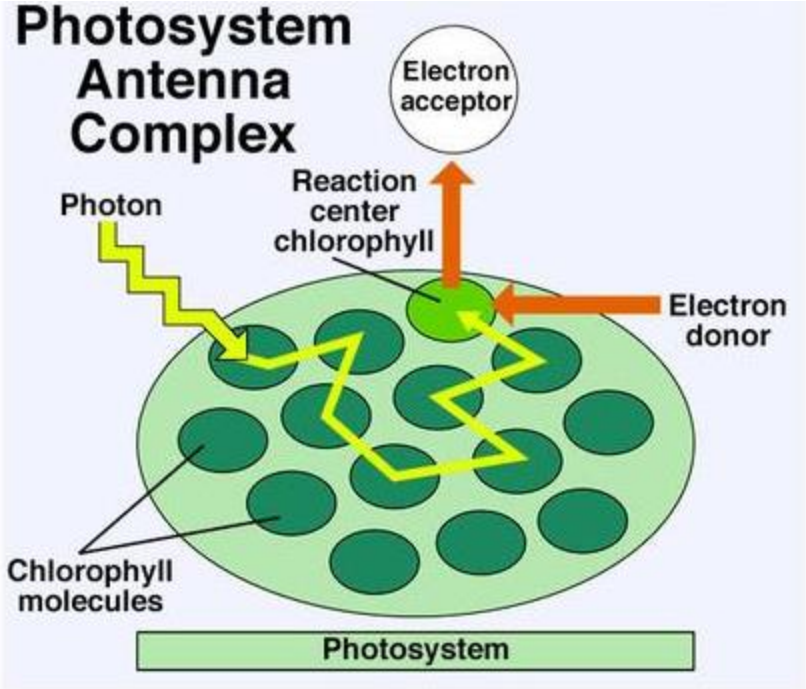

Figure 4: Photosystem Antenna Complex (McGraw-Hill)

The antenna pigments are predominantly chlorophyll *b*, xanthophylls, and carotenoids. Chlorophyll *a* is known as the core pigment. Except of chlorophyll, other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthin in brown algae and diatoms resulting in a wide variety of colors. Their absorption spectra are non-overlapping in order to broaden the range of light that can be absorbed in photosynthesis. The carotenoids have another role as an antioxidant to prevent photo-oxidative damage of chlorophyll molecules. According to the literature blue and red light provide higher chlorophyll content, while lower chlorophyl production is observed in cultures under green light. This is correlated with the fact that chlorophyll *a* has a maximal absorption for blue and red wavelengths, whereas little absorption occurs for green wavelength (Figure 5). Furthermore, Jahns and Holzwarth 2012 found out that carotenoids such as β-carotene and lutein are important in PS II. They harvest blue light and transfer the energy to photosystem reaction center. As a result carotenoids protect the photosynthetic apparatus against photooxidative damage by deactivating reactive oxygen species (ROS) and reducing the ROS formation under excess light. ROS are produced as a consequence of electron transport processes in photosynthesis and aerobic respiration. ROS, as their name suggests, are reactive and potentially harmful to cells, causing oxidation of lipids, proteins and DNA. High levels of ROS production lead to a process that is often referred to as “oxidative stress”. (Ravelonandro et al. 2008)

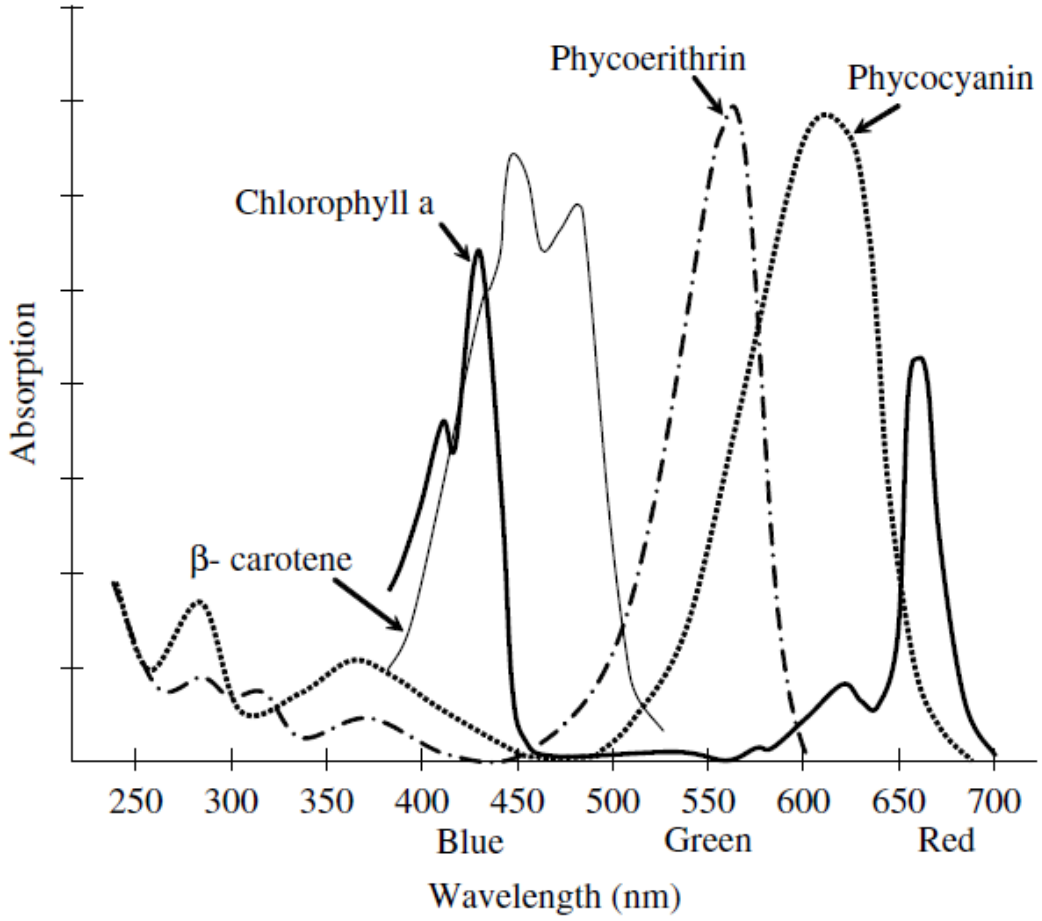

Figure 5: Absorption spectrum of several pigments of cyanobacteria (Ravelonandro et al. 2008)

Despite light is a requirement for growth of photoautotrophic microalgae, oversaturating light can cause photo-oxidation and cell damage (Rise et al. 1994). Microalgae are able to absorb photons only in the photosynthetic active radiation (PAR), light wavelengths from 400 to 700 nm. Photons can be absorbed as energy source. Both light intensity and quality (colour or wavelength) influence the light use efficiency. Mohsenpour et al investigated the effect of light conditions on the growth of green algae. He filtered different wavelengths of visible light containing violet, green, orange and red and found that the highest specific growth rate was achieved using orange range. Moreover he examined the pigment production and found that red and green light result in maximum chlorophyll α production for different green algae (Mohsenpour et al. 2012). Microalgae grown under high irradiance show lower photon use efficiencies than microalgae grown under low irradiance (Mohsenpour & Willoughby 2013). The antenna chlorophylls are forced to absorb more photons than can be processed biochemically. Therefore, in case of exposure to high irradiance, these photons are dissipated as heat and fluorescence. According to this theory, in a microalgae culture the thin layer of reactor volume close to the reactor surface absorbs a large percentage of the incoming photons, while at the back of the culture this additional light energy could have been used efficiently (Melis et al. 1999). This problem leads to low light use efficiency in the culture and causes a reduction of the biomass productivity as compared to maximum theoretical productivities (Formighieri et al. 2012).

To respond in changing light conditions microalgae use state transition, which is a rapid mechanism for reconfiguring the photosynthetic light-harvesting apparatus. When photosystem II is more excited than photosystem I the transition to State 2 is induced, in which more energy is transferred to PSII. When

PSI is over excited compared with PSII transition to State I is induced and more energy is transferred to PSI. (Murata 1969)

The fraction of light that microalgae absorb depends on the wavelength specificity of the different pigments, and the concentration of these pigments. This fraction can be calculated from the absorption coefficient and is different for each light colour because it depends on wavelength and the acclimation state of the microalgae. Algae can anticipate on the experienced light conditions changing the amount of pigments in order to absorb more or less light, which is called photo acclimation. Thus, when a culture is cultiaved under low light intensity, they increase the amount of pigments or, in other words, the optical cross section so that they can absorb more light energy per cell. On the other hand, when they are experiencing a high light intensity, they decrease their pigmentation in order to prevent oversaturation. (Janssen & Lamers 2013)

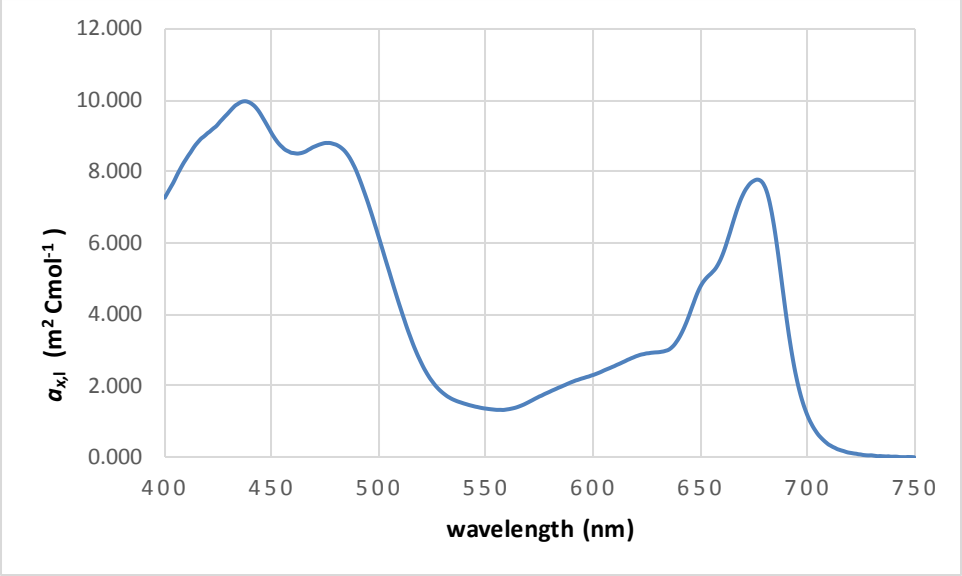

Figure 6: Optical cross section of C. reinhardtii in white light(Janssen & Lamers 2013)

In many of the photosynthetic organisms, the photosynthetic saturation level is at approximately 25% of the full solar light intensity, which means that any additional absorbed light is dissipated as heat or fluorescence (Blankenship & Chen 2013). There are several options to prevent this waste of light energy. One approach is to genetically engineer the microalgal cells in order to reduce the size of their antenna complexes (Mussgnug et al. 2005) (Kwon et al. 2013). To mimic this mutant behavior one option is the use of different light colours, like green. In a culture exposed to green light, only a small light fraction will be absorbed, and the rest will penetrate deeper into the culture. It is known that green light, at high light intensities, can penetrate deeper in the culture and drive photosynthesis more efficiently than the other colours. In this way, a higher volumetric productivity can be achieved (Terashima et al. 2009).

Fv/Fm ratio

Fv/Fm is the most used chlorophyll fluorescence measuring parameter in the world and tests whether plant stress affects photosystem II in a dark adapted state. When light is absorbed, it can follow three pathways. The first one is to be used in photosynthesis and produce ATP and NADPH, the second option is to be re-emitted as fluorescence and the third to be dissipated as heat. The Fv/Fm test is constructed to force the maximum amount of light energy to follow the fluorescence pathway. Fo is

the minimum fluorescence, in the pre-photosynthetic fluorescent state when algae are dark-adapted. Fo is measured using too low light source to drive to photosynthesis. Fm is the maximum fluorescence, when the maximum number of reaction centers have been reduced or closed by the oversaturating light. Fm is measured when the algae sample is exposed in an intense light flash, which closes all reaction centers. The difference between maximum and minimum fluorescence is Fv and is called variable fluorescence. The greater the algae stress, the less open reaction centers available and as a result the Fv/Fm ratio is lower. Fv/Fm ratio indicates the mazsimum potential quantum efficiency of photsystem II, when the reation centers are all open. (Baker 2008)

Materials and methods

Organism, medium and cultivation conditions

Chlamydomonas reinhardtii CC1690, obtained from the Chlamydomonas Research Centre, was cultivated first in 250ml shake flasks containing 100ml medium (Table 1) at pH 6.7 The composition of *Chlamydomonas reinhardtii* medium was designed to reach 6 g/L biomass dry weight. The recipe for the medium can be found in the appendix. Medium sterilization was done by filtering through a Sartorius liquid filter (0.2µm pore size) in a sterilized vessel. The cultures were placed in a cultivation room, which had a constant temperature of 25°C and light intensity 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The medium in photobioreactors for turbidostat and chemostat experiments was enriched with extra 0.4 g L^{-1} urea to ensure high growth of microalgae. For the cultivation of *Chlamydomonas reinhardtii* on plates the same medium was used with the addition of 15 g L^{-1} Agar.

Table 1: Medium composition

Medium		**Trace Element**	
Macronutrients	**final concentration (g L^{-1})**	**Micronutrients (Hütner)**	**final concentration (g L^{-1})**
KH_2PO_4	0.706	$FeSO_4 \times 7H_2O$	0.01
K_2HPO_4	1.465	$Na_2EDTA \times 2H_2O$	0.1107
$MgSO_4.7H_2O$	0.560	$ZnSO_4.7H_2O$	4.40E-02
$CaCl_2.2H_2O$	0.114	H_3BO_3	2.28E-02
$CO(NH_2)_2$	0.99	$MnCl_2.4H_2O$	1.01E-02
		$CoCl_2 \times 6H_2O$	3.22E-03
		$CuSO_4.5H_2O$	3.14E-03
		$(NH_4)_6Mo_7O_{24} \times 4H_2O$	2.20E-03

Reactor set up and experiments

Chlamydomonas reinhardtii was cultivated in pre-sterilized (30 min at 121°C) photobioreactor (Algaemist, mechanical workshop Wageningen UR) which has a volume of 0.4L, a lightpath of 1.4cm and an irradiated surface area of 0.028 m^2. The culture temperature was at 25 ˚C, the pH was at 6.7 (± 0.1) with 200 mL/min airflow. Antifoam solution 1 % (v/v) (Antifoam B, Mallinckrodt Baker B.V., the Netherlands) was being pumped in the reactor for 1 min every 2 hours in the beginning of the experiment when the biomass concentration was low and for 2 min per 2 hours when the biomass concentration was much higher, which was 10 – 20 ml per day.

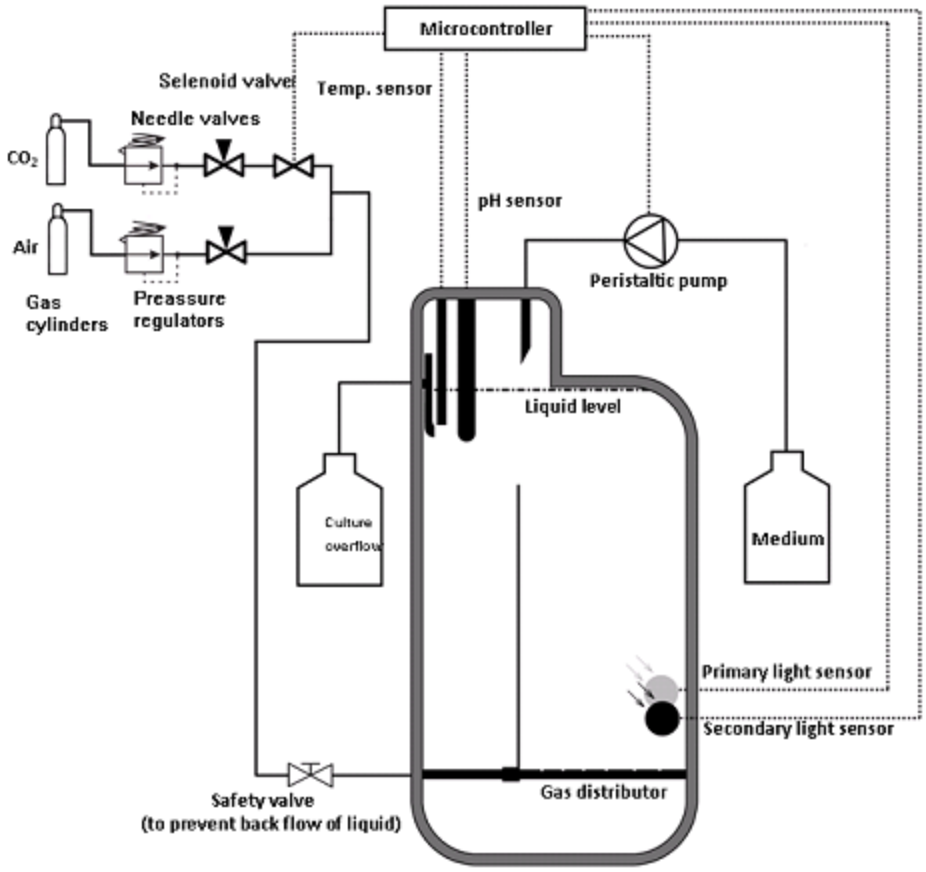

Figure 7: Algaemist flat panel photobioreactor for chemostat and turbidostat experiments. There are pH sensor, temperature sensor and secondary light sensor, which are connected with the microcontroller and secure the optimal conditions for microalgae cultivations. Moreover, Algaemist is connected with CO_2 and air supply that flow into the reactor through a gas distributor.There are two pumps, one for medium and one for antifoam, and a vessel for culture overflow.

Model estimation of volumetric productivity

A productivity model were used in order to predict the volumetric productivity (r_x), the biomass concentration (C_x) and the biomass specific growth rate (μ). This model was constructed to calculate these values according to specific parameters about photobioreactor and *Chalmydomonas reinhardtii* strain. For a detailed description of the model calculations please refer to appendix.

Volumetric productivity measurements

Turbidostat experiments were done to determine the volumetric biomass productivity. After the inoculation the reactor was started running in batch mode until the biomass concentration reach the preferred value in order to have an outgoing light intensity of 10 μmol m^{-2} s^{-1}. The ingoing light intensity was being increased every day for 3 days until an ingoing light intensity of 1500 μmol m^{-2} s^{-1} and an outgoing light intensity of 10 μmol m^{-2} s^{-1} were obtained. The ingoing light intensity the first day was around 100 μmol m^{-2} s^{-1}. This outgoing light intensity was chosen because is the minimum light intensity needed for maintenance of the algae. If the outgoing light intensity is less than 10 μmol m^{-2} s^{-1} the culture at the back of the reactor is in dark zone, the light is limited, which has negative effect in the productivity. Tubidostat mode ensured a constant light regime, under different light colours (blue, red, amber and amber-blue). Daily measurements of dilution rate and biomass concentration were conducted, until when at least 5 measurements with stable values were obtained. Each

photobioreactor was connected to a harvest vessel via the overflow. Samples from these vessels were taken every day at a specific time in order to measure the dry weight.

Maximum yield on light measurements

The maximal yield of biomass was measured in a chemostat mode under blue and white light at low light intensities and dilution rates between 0.013 h^{-1} and 0.053h^{-1}.

The specific growth rate (μ) could be measured because in a chemostat in steady state it is assumed to be equal to the dilution rate. Moreover, the following equations were used that correlates μ (h^{-1}) to the yield of biomass per photon ($Y_{x/ph,m}$) (g mol^{-1}) and the maintenance requirement of algae (r_m) (mmol g^{-1} h^{-1}). (Janssen & Lamers 2014)

$$\mu = Y_{x/ph,m} \cdot q_{ph} - r_m \quad (5)$$

Where q_{ph} (mol s^{-1} $Cmol^{-1}$) is the specific photon consumption rate which can be calculated using the following equation:

$$q_{ph} = \frac{I_{ph,in} - I_{ph,out}}{C_x \cdot d} \quad (6)$$

Where C_x (g L^{-1}) is the biomass concentration and d (m) is the reactor depth. By measuring μ and q_{ph} a plot of q_{ph} versu µ can be constructed, where the intercept of the graph is the $-r_m$ value and the slope is equal to $Y_{x/ph,m.}$

Different ingoing light intensities were selected for white and blue light in order to have as high as possible photon consumption rate but still low enough to prevent oversaturation. The white and blue reactor should have the same biomass specific light absorption rate equal to 284.8 mol s^{-1} $Cmol^{-1}$, in order to have fair comparison of the yields, thus incident light intensities of 82 µmol m^{-2} s^{-1} and 32 µmol m^{-2} s^{-1} respectively were calculated according to equation 7.

$$a_x \cdot I_{ph} = q_{ph} \quad (7)$$

where a_x (m^2 $Cmol^{-1}$) is the biomass specific absorption coefficient and I_{ph} (µmol m^{-2} s^{-1}) is the light intensity. q_{ph} and a_x are known for each light colour and I_{ph} can be calculated.

Biomass concentration

The dry weight biomass concentration was determined after taking fresh samples from the reactor and the overflow, almost 1mg biomass per filter. A Buchner flask and a Whatman filter (55mm diameter and 0.7 µm pore size) were used to filter the sample of microalgae diluted with 50 ml demineralized water under mild vacuum. Just before filtration the filter was washed with demi water. The empty filters first were dried overnight at 95°C and then placed in a desiccator to cool to room temperature. Before filtration the filters were weighted and wetted with demineralized water. After filtration, the wet filters that contain the samples were dried at 95°C overnight. Then the dry filters were placed in a desiccator for 1 hour and weighted. Finally, the filters were weighted on accurate scales and the biomass in g L^{-1} were calculated.

Fv/Fm ratio

The analysis is conducted using an Aquapen AP 100 (Photon Systems Instruments, Brasov, Czech Republic). The samples of the reactor exposed to amber coloured light contained 60 µl sample and

1.94 ml tap water. All other samples contained 120 µl sample and 1.88 ml tap water. Before the measurement, the samples were placed in the dark for 20 minutes.

Absorptive cross section

Everyday samples were taken directly from the reactor for spectrum analysis with a double beam spectrophotometer (UV-2600, Shimadzu, Japan). 2 mm light path cuvettes with 2 ml tap water as blank and 1ml (diluted) or 2 ml (undiluted) samples were used. The samples from a reactor under amber light were diluted because the biomass concentration was too high, and the microalgae were creating sediment into the cuvette. The spectrophotometer measures the wavelength dependent absorbance between 300 and 750 nm. The Lambert-Beer equation was used in order to calculate the absorption coefficient a_x^{DW}($m^2\ g^{-1}$).

$$a_x^{DW} = \frac{A \cdot LN(10)}{C_x \cdot l} \tag{8}$$

Where Cx is the biomass concentration in $g\ m^{-3}$ and l the light path in m.

Results and discussion

Predicted volumetric productivity

Using a simple kinetic model we predicted the volumetric productivity (r_x), the biomass concentration (C_x) and the biomass specific growth rate (μ) for different light colours. For a detailed description of the model calculations please refer to appendix growth model. It was estimated that for the alga *Chlamydomonas reinhardtii* cultivated at high light intensities (1500 µmol $m^{-2}s^{-1}$), the biomass productivity using amber-coloured light will be higher compared to the other colours, such as blue light, where the productivity will be lower (Table 2). Figure 8 illustrates that by combining the absorption coefficient and light intensity (equation 7), the highest and lowest biomass specific light absorption rate is observed for blue and amber light respectively. It is important to mention that these differences between colour are in the first layers of the reactor, while when the light pnetrates deeper in the reactor these differences are getting lower. Thus, a microalgal culture will get oversaturated easier under high intensities of blue light than under amber light.

Table 2: Estimated biomass concentration, productivity and specific growth rate according to the model

	Peak wavelength (nm)	Cx (g L^{-1})	r_x (g $L^{-1}d^{-1}$)	µ (1 d^{-1})
Sunlight		2.2	3.5	1.6
Amber light	600±75	2.5	4.5	1.8
Warm-white light	400-700	2.4	4	1.7
Blue light (450nm)	460±50	0.8	1.7	2.2
Deep red light (660nm)	660±20	1.2	2.5	2
Orange red light	640±50	1.7	3.3	2

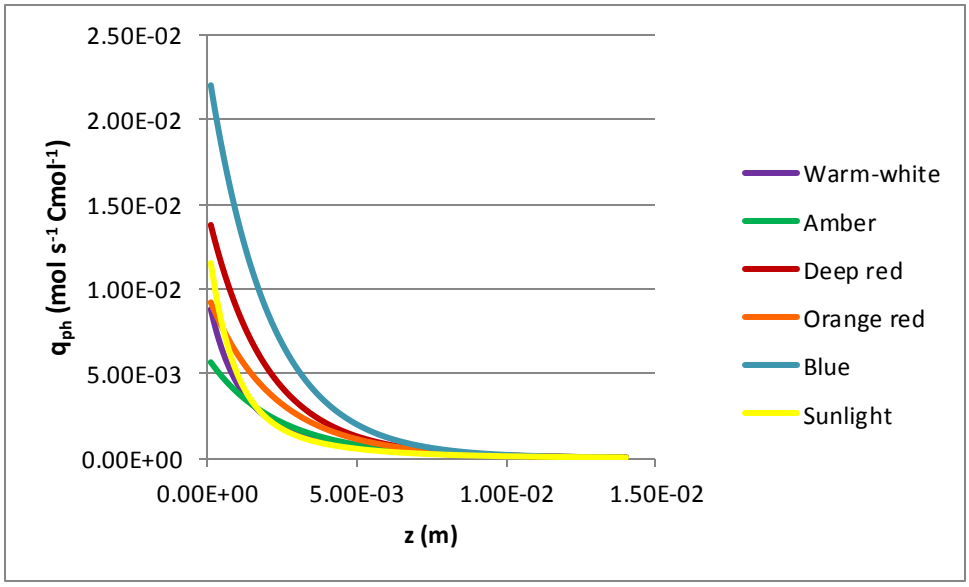

Figure 8: Biomass specific light absorption rate for different colour versus reactor depth z.

Volumetric productivity

In a series of experiments the volumetric productivity of Chlamydomonas reinhardtii was measured under different light colours at high light intensities, 1500 µmol m^{-2} s^{-1} (Figure 9). The experiments under orange red and white light and some series for the other colours were conducted by another student Guus de Vries. He used the same experimental set-up and light intensities. The only difference was that for the medium of Guus experiments 0.99 g/L urea were used, while as it mentioned in

Materials and Methods section our medium was enriched with extra 0.4 g L^{-1} urea. The results of Guus were used for two reasons. First to support and give a better explanations of the results of this report and second because we didn't have stable cultures under orange led light and cultures under white light. We tried to cultivate our strain under orange-red light more than 5 times, but the culture were not stable for more than 2 days in high light intensity. Guus found that cultures under deep red and blue light resulted lower volumetric productivity than cultures under white light. Moreove r he had non-stable cultures under amber light but there is an indication that amber light can result in higher volumetric productivity. Figure 9 illustrates that cultures under deep red and blue light, 1.25 g l^{-1} d^{-1} and 1.97 g l^{-1} d^{-1} respectively, present lower productivity than white light, 3.52 g l^{-1} d^{-1}. This result confirms the hypothesis that higher light absorption rate per cell leads to lower productivity. In addition, for the deep red light experiments under 1500 µmol m^{-2} s^{-1} deep red light the results were not stable, thus the light intensity was decreased to an average value of 838 µmol m^{-2} s^{-1}.

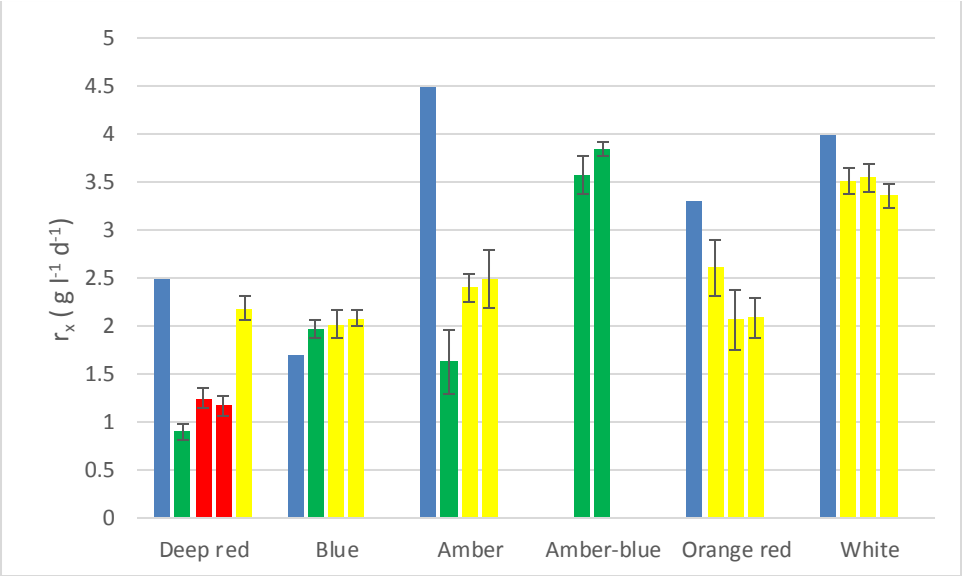

Figure 9: Volumetric productivity (r_x^{μ}) of continuously cultivated microalgae exposed to deep red, blue, amber, amber-blue, orange red and white coloured light. The blue bar is the volumetric productivity as predicted by the microalgal growth model. Each bar illustrates the average value of at least 5 daily measurements. Green bars indicate experiments conducted at high light intensities under different colours. Red bars illustrates experiments conducted under deep red at lower light intensity 838 µmol m^{-2} s^{-1}. Yellow bars represent experiments conducted at the same high light intensities by Guus de Vries.

On the other hand, Figure 9 shows that productivity for all colours is lower than the predicted according to the model, but specifically for amber light this off-set between experimentally and predicted values was the biggest. So we repeated the experiment with the addition of 50 µmol m^{-2} s^{-1} of blue light. We chose blue light because it is the best absorbed light col our and with this addition we tried to keep the culture as much healthier. Unfortunately there was not much time to combine other light colours as well for algae cultivation. As already mentioned in theory blue light provide s more chlorophyll content than the other colours. Moreover it was mentioned in theory that β-carotenes that absorb only blue light carotenoids protect the photosynthetic apparatus against photooxidative damage. The amount of blue light that was added was not too much to affect the productivity. According to the model microalgae under 50 µmol m^{-2} s^{-1} of blue light present volumetric productivity 0.35 g l^{-1} d^{-1}. It is important that the combination of blue and amber light has as a result more than a doubling in volumetric productivity (3.85 g l^{-1} d^{-1}) compared to only amber light and is the highest among the other colours. Productivity for amber blue light is even higher than for white one and it is statistically significant ($P<0.01$). That confirms our hypothesis that lower light absorption rate per cell results in higher volumetric productivity. It is obvious that addition of blue light has an enormous

biological effect, because 50 µmol m^{-2} s^{-1} as energy level are too low to double the productivity. Furthermore, in the deep red light, the bar graph shows that cultures under lower light intensities (red bars) have higher volumetric productivity (1.25 g l^{-1} d^{-1} and 1.17 g l^{-1} d^{-1}) than under high light intensities (green bars) (0.9 g l^{-1} d^{-1}). This difference is statistically significant ($P<0.001$). It is interesting that there is only a small difference, which means that the yield on light at high light was very low. The results of the current study and Guus results were not different for blue reactor ($P>0.05$), but they were different for deep red ($P<0.001$) and amber reactor ($P<0.05$). One explanation could be that algae cultures under amber light were not stable in this study nor in Guus experiments. This makes the results for amber reactor not reliable. It is important to mention that all experiments were stopped when flocculation appears in the culture. This might have been caused by the antifoam solution which was added in the rector. It is known that antifoam could have negative influence in a microalgae culture but the concentration that it would be harmful is not clear.

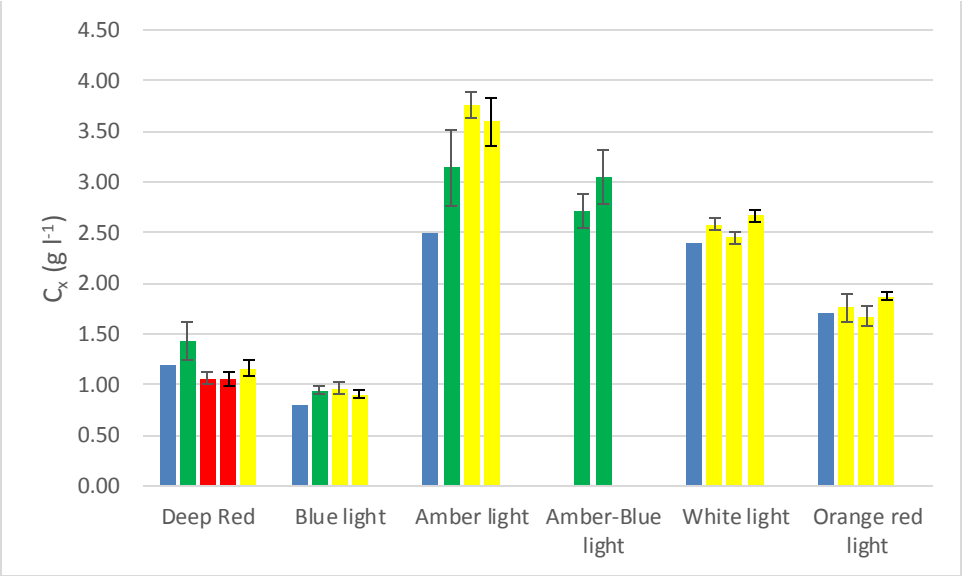

Figure 10: Biomass dry weight content for deep red, blue, amber, amber-blue, white and orange red light. The blue bar is dry weight predicted by the productivity model. Each bar illustrates the average value of at least 5 daily measurements. Green bars indicate experiments conducted at high light intensities under different colours. Red bars illustrates experiments conducted under deep red at lower light intensity 838 µmol m^{-2} s^{-1}. Yellow bars represent experiments conducted at the same high light intensities by Guus de Vries.

Figure 10 depicts that dry weight is highest for amber and amber-blue reactor, 3.14 g l^{-1} and 3.05 g l^{-1} respectively, compared to deep red and blue reactor, 1.43 and 0.94 g l^{-1}. Moreover in this graph the experimentally obtained dry weight measurements are in line with the predicted values by the microalgal growth model. Values of dry weight is the easiest value for the model to predict because there are not so many assumptions to make. This graph verifies the main hypothesis of this report that the higher the absorption rate per cell the lower the productivity is. In all cultures 1490 µmol m^{-2} s^{-1} were absorbed, however for amber one a lot of biomass was produced in order to absorb all this light. As a result there is less light absorption per cell. This supports the hypothesis that light colours can be used to simulate antenna mutants. The dry weight for deep red and blue reactor was lower than the other colours because these lights are better absorbed and in high light intensities they cause oversaturation to the photosystem and a low light use efficiency.

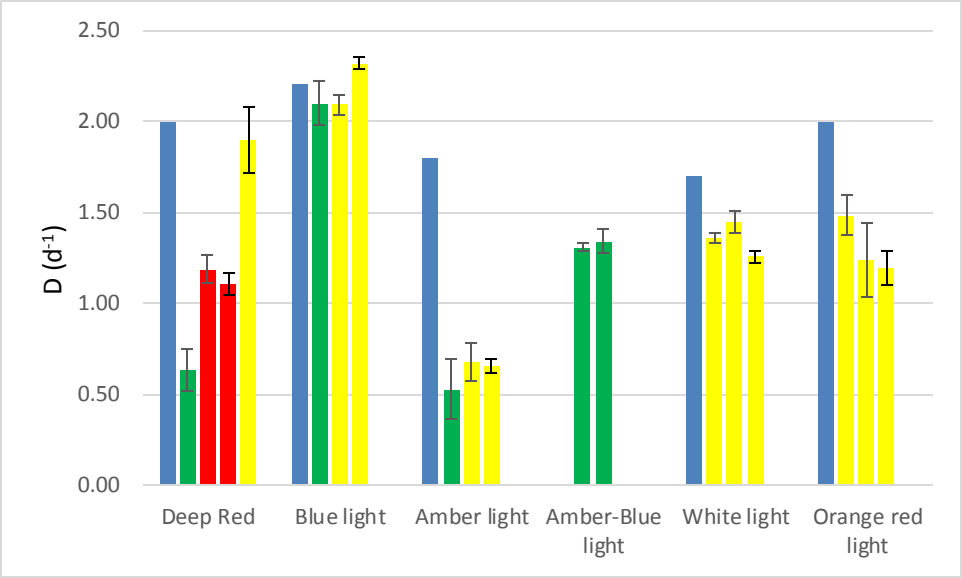

Figure 11: Dilution rate (D) for deep red, blue, amber and amber-blue light. The blue bar is the dilution rate as predicted by the microalgal growth model. Each bar illustrates the average value of at least 5 daily measurements. Green bars indicate experiments conducted at high light intensities under different colours. Red bars illustrates experiments conducted under deep red at lower light intensity 838 µmol m^{-2} s^{-1}. Yellow bars represent experiments conducted at the same high light intensities by Guus de Vries.

According to mass balance in a turbidostat experiment dilution rate is equal with the specific growth rate of the microalgae. Figure 11 shows that the specific growth rate is the highest for blue light, 2.2 d^{-1}, compared to the other lights. That shows that blue light is well absorbed by a small amount of biomass, and therefore they are alla saturated and grow extremely fast compare to the other colours. The most important discovery is that the addition of blue light in amber reactor result the increase of specific growth rate from 0.53 d^{-1} to 1.34 d^{-1}, which shows that this addition induces a biological response of the cell. Yan and Zheng found that blue light wavelengths can promote microalgal metabolism by inducing PS I (Yan & Zheng 2014). β-carotene and lutein harvest blue light and transfer the energy to photosystem reaction center. As a result under blue light microalgae can photosynthesize better and have higher growth rate.

As figure 15 shows the measurements for the amber light exposed reactor were not stable enough and Guus faced the same problem under this colour. One explanation is that the antifoam solution that was added was not constant or precise and maybe it was toxic for the culture. This explanation is not supported by the other results because under other colours the microalgae cultures were stable enough. The most stable culture was under blue light (Figure 14) which lasted 10 days before flocculation started. Therefore we added a small fraction of blue light to the amber reactor to obtain a more stable cultivation. The result was a more stable culture compare to the one under only amber light. It is important that in a run of experiments a culture under combination of blue and amber light was stable for 9 days and the 10th day the culture started flocculating. In addition, for the deep red light experiments under 1500 µmol m^{-2} s^{-1} deep red light the results were not stable (Figure 12), thus the light intensity was decreased to an average value of 838 µmol m^{-2} s^{-1}. However, the culture under deep red light was more stable when the light intensity was decreased to 838 µmol m^{-2} s^{-1} (Figure 13). On the contrary Guus manage to have a stable culture of microalgae under deep red at 1500 µmol m^{-2} s^{-1}. Furthermore Guus had stable cultures under orange-red light as well, something that was impossible in our experiments and our cultures under orange-red were being flocculated after the second day at high light intensities.

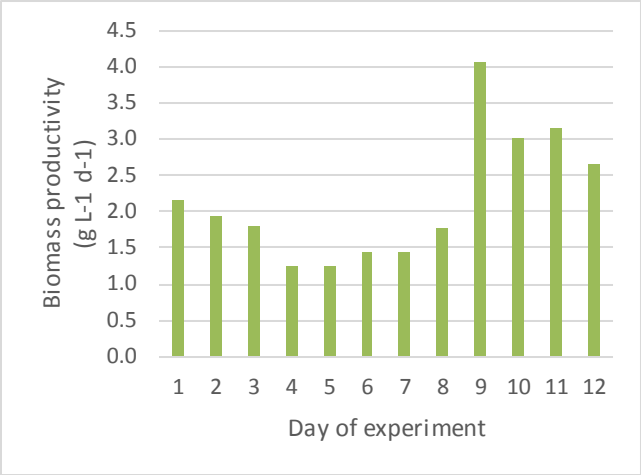

Figure 15: Volumetric productivity for cultivation under amber light.

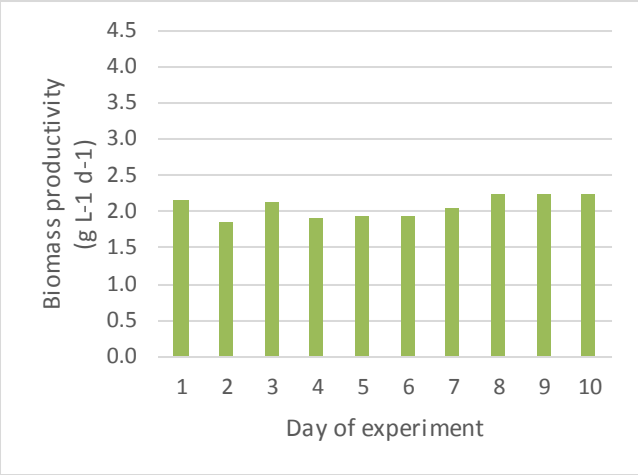

Figure 14: *Biomass productivity for cultivation under blue light.*

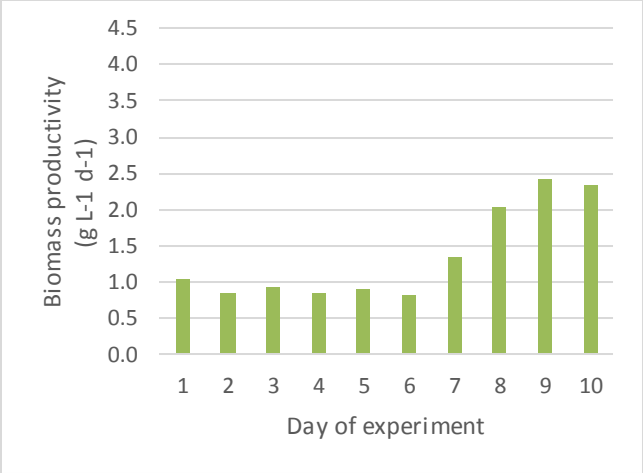

Figure 12: Biomass productivity for cultivation under deep red light of 1500 µmol m^{-2} s^{-1}.

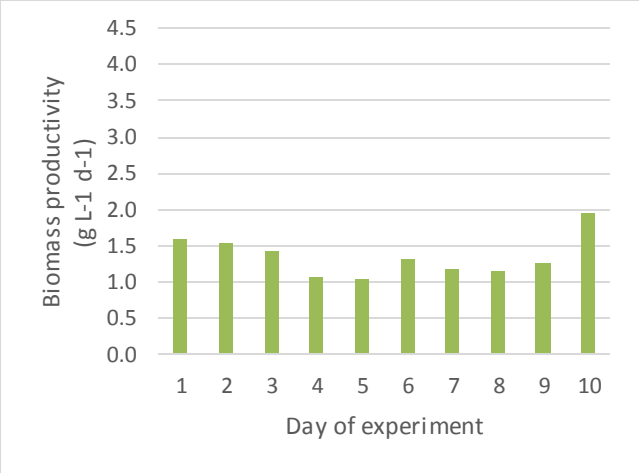

Figure 13: Biomass productivity for cultivation under deep red light of 838 µmol m^{-2} s^{-1}.

As it was already indicated, except for the blue reactor, all the cultures show lower values for volumetric productivity (1.97 g L^{-1} d^{-1}) than the value that was predicted, 1.7 g L^{-1} d^{-1} by the model. However, the experiments in the blue reactor showed slightly higher volumetric productivity and dry weight and comparable dilution rate with the predicted values according to the model. These differences are caused probably by wrong assumptions in estimations and calculations of the model. For instance in the model there is the assumption that the maintenance coefficient is constant for the culture, something that is not true in real experiments. Also the model uses the assumption that all ingoing light intensity is used by microalgae. In reality there is the phenomenon of back scattering in the reactor and not all light intensity is going through the reactor. Furthermore, a model is based on ideal and stable conditions, something that is difficult to realize in practice. Moreover the ingoing light intensity that we measured and the model used and the actual light intensity were slightly different because we measured the light intensity on the surface of the reactor with the help of a dummy reactor. Using this dummy reactor the light intensity is measured in 27 spots on the surface of the reactor and it may be different than the true average ingoing light intensity. In addition, inside the

reactor there is a magnet that is used in order to clean the glass of the reactor from the microalgae glass growth. This magnet blocks a bit of the ingoing light intsity.

It is difficult to compare productivity results with other research performed in this field because there are not many experiments with *C. reinhardtii* under high light intensities. *Chlorella sp.* (Zhao et al. 2013), *Scenedesmus sp*. (Kim et al. 2014) Spirulina platensis (Wang et al. 2007) and *Botryococcus braunii* (Baba et al. 2012) showed higher productivity when they were cultured under red light than under blue or white light. Moreover Das and his team found that *Nannohloropsis sp.* presents higher light energy conversion to biomass when it is illuminated with blue light than with red light (Das et al. 2011). Unfortunately these results are not comparable because all these experiments were conducted in low light intensities (50 µmol m^{-2} s^{-1}). In these low light intensities light absorption is the limiting factor and not the absorption efficiency that this report investigates. However Cuaresma et al. (2009) cultivated *Chlorella sorakiniana* at 2100 µmol m^{-2} s^{-1} of orange-red light and the biomass yield on photons reached 1 g mol^{-1}. It could be due to the different strain that they used and the different dilution rate because the experiments were conducted under a variety of dilution rates.

Results for dry weight deviate from what can be found in the literature. Cheng Yan and his collaborators determined that the order of highest dry weight production for the *C.vulgaris* reproduction is red, white, yellow, purple, blue and green in 2000 µmol m^{-2} s^{-1} (Yan et al. 2013). These experiments we conducted in shakeflasks, in batch reactor, thus it is not reliable to compare these results with the results of your turbidostat experiments. However Jeong et al. (2012) found that light with shorter wavelengths, such as blue, green and purple are more efficient in photosynthesis.

The dilution rate values are higher than the literature results and not comparable with them. Mohsenpour & Willoughby (2013) found that the dilution rate for C. vulgaris was 0.5 d^{-1} and 0.19 d^{-1} for green light in low and high density cultures respectively at 250 µmol m^{-2} s^{-1}. This is way lower than the light intensities that used for this report. For the other colours blue light has higher dilution rate in low density cultures and red one for high density cultures. This literature is not comparable with our results because the experiments conducted under only 20 µmol m^{-2} s^{-1}, which is much lower intensity.

In addition, Guus de Vries (2014) conducted the same experiments and found that the volumetric productivity was for lower orange red, deep red and blue light than for white light. These results are included in the graphs of this study because they are similar to our results and support our hypothesis. However he found that amber light shows lower volumetric productivity than white light, something that is not expected. We had the same result for the amber without the addition of blue light. A possible explanation could be that he also faced difficulties to keep the culture under amber light in steady state. Moreover, Guus found that the culture under amber light produced the highest biomass concentration and under blue light the lowest concentration compare to the other colours. This conclusion is similar to our and indicates that culture under amber light has to produce more biomass in order to absorb the same light with the cultures under different colours. Hence amber reactor presents less light absorption rate per cell. Furthermore, his results for the dilution rate are same as our, because the highest and lowest dilution rate was investigated under blue and amber light respectively.

Fv/Fm

Every day fresh sample from the reactors was taken directly to determine the Fv/Fm ratio, which is chlorophyll fluorescence measuring parameter and tests whether plant stress affects photosystem II in a dark adapted state. In other words Fv/Fm ratio is a measure for the maximal quantum yield. For

each culture the average of the daily measurements and the standard deviation were calculated (Figure 18).

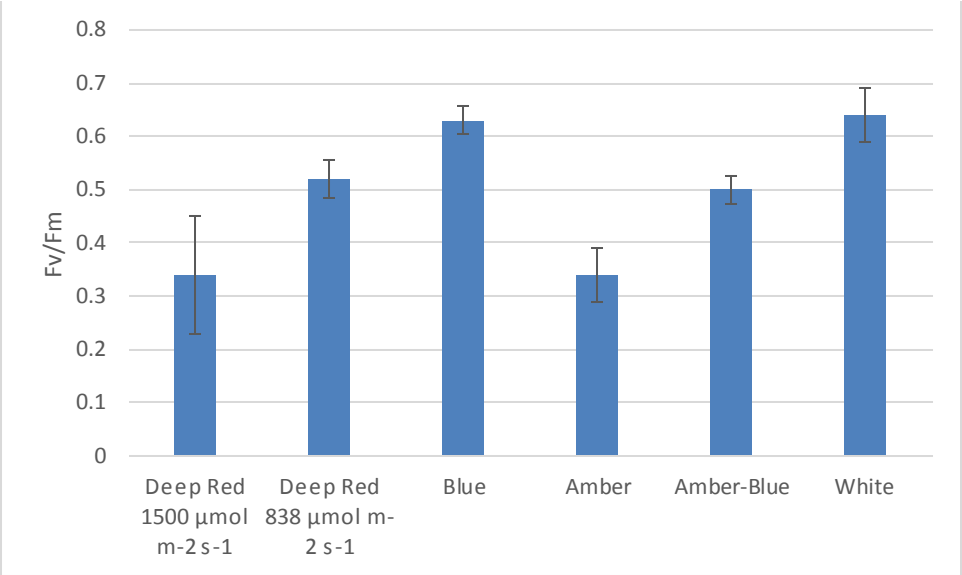

Figure 16: Fv/Fm measured for samples from algae cultures under 5 different colours of light (White, Amber-blue, Amber, Blue and Deep red). The measurements for white light were taken from a thesis report of another student (Guus de Vries).

As figure 16 shows the amount of light energy per cell has effect on the Fm/Fv ratio. It is illustrated that algae cultivated under white and blue light have the highest Fm/Fv ratio compared to the other colours, 0.64 and 0.63 respectively. Moreover, another important result is that cultures under combination of amber and blue lights have higher Fm/Fv ratio than the cultures under only amber light. This difference is statistically significant ($P<0.0001$). This was not expected, because according to theory the addition of blue light would result in more saturation and thus a lower Fv/Fm ratio. The low values for amber reactor could be caused by the lower dilution rate and as a result more old cells in the reactor. Furthermore, in the graph the values for deep red light are lower for higher light intensities, which means that the photosystem II is damaged while in lower light intensities the value is higher, that shows that the microorganism is healthier. The difference between deep red in high and lower light intensities is statistically significant ($P<0.05$).

If a photosystem II in algae functions properly the Fv/Fm ratio should be around 0.7 (Simis et al. 2012) . Cultures under white and blue light showed Fv/Fm ration in range of 0.58-0.68, so the photosystem II of the culture is more efficiency compare to the cultures under other light colours. There are 2 possible explanation for the lower Fv/Fm ratio of the other colours except blue. First, low Fv/Fm in healthy cells can be a measurement artefact when the light source does not provide sufficient intensity to saturate PSII (Raateoja et al. 2004). We checked this explanation, by measuring Fv/Fm ration in algae under even higher light intensities and it was not responsible for low Fv/Fm ration. The second one is that high irradiance is a stress leading to photoinhibition. When algae are grown in different light conditions, the photosystem II responses different to increasing light intensities. Algae acclimated in low light intensity present low capacities for photoprotective responses and photosynthetic electron transport. Thus these algae become photoinhibited by the sharply increase of light intensity (Demmig-Adams & Adams 1992). In our case the light intensity of 1500 µmol m^{-2} s^{-1} is probably too high for deep red light and damaged the photosystem II of algae causing photoinhibition. This hypothesis is supported by the experiments for deep red light of high and low light intensity, which shows that the culture under lower deep red light has much higher maximal quantum yield. Furthermore, Tamulaitis et al. (2005) support our hypothesis, because they investigated that far red light influence the physiological processes up to complete breakdown of photosynthesis. Moreover, 1500 µmol m^{-2} s^{-1} of blue light are oversaturating the photosystem, but it looks like blue light activates photoprotection. Fu et al. (2013) state that in a culture of *Dunaliella salina* blue light results in non-photosynthetic

quenching, while it generates reactive oxygen species. This happens because photons of blue light have higher energy than that photosynthesis requires. Hence algae accumulate photosynthetic pigments, such as xanthophylls in order to protect photosynthesis apparatus from reactive oxygen species. In addition the light signal transduction of blue light may be different from that of red light since plants usually have different photoreceptors and domains.

Yield of biomass on light energy

The biomass yield on light energy was calculated for the microalgal cultures under 4 different colours (Figure 17).

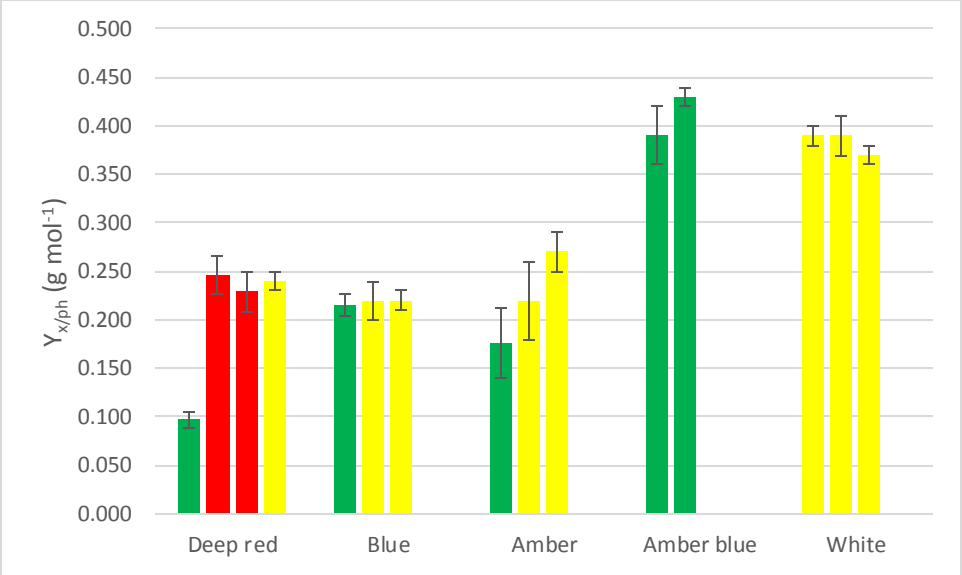

Figure 17: Biomass yield on light for 5 different colours of light (Deep-red, blue, amber, amber-blue and white light). Green bars indicate experiments conducted at high light intensities under different colours. Red bars illustrates experiments conducted under deep red at lower light intensity 838 µmol m^{-2} s^{-1}. Yellow bars represent experiments conducted at the same high light intensities by Guus de Vries.

First of all, figure 17 illustrates that microalgae under combination of blue and amber light present higher biomass yield on light energy than the other 4 colours, equal to 0.43 g mol^{-1} and this difference is statistically significant ($P<0.05$). Guus de Vries found that white light presents the highest productivity among the other colours. Moreover, deep red reactor in 1500 µmol m^{-2} s^{-1} has lower biomass yield than in 838 µmol m^{-2} s^{-1}, 0.098 g mol^{-1} and 0.246 g mol^{-1} respectively. These values for red reactor in lower light intensities are similar with Guus results. Furthermore, for the amber reactor the biomass yield on light increased 2 times when we added a small percentage of blue light and it is statistically significant ($P<0.01$). As for the amber light experiments Guus results were statistically significant higher that the results of the current study ($P<0.5$). This happe ned because it is difficult to achieve a stable culture under amber light, something that is indicated by the standard deviation in figure 17.

Spectrum analysis

As it is already mentioned, every day fresh sample was taken directly from all the reactors in order to measure the absorption spectrum. These values were measured by a double beam spectrophotometer and were used to determine the mass absorption coefficient. Figure 18 demonstrates the mass

absorption coefficient (a_x^{DW}), which was calculated according to equation 8, against the wavelength for 5 light colours.

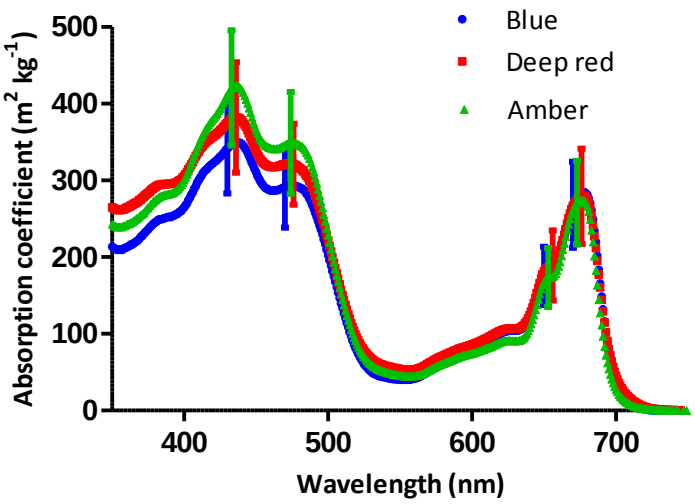

Figure 18: Average mass absorption coefficient of at least 4 measurements for 3 different colours (Blue, Deep red and Amber blue). Standard deviations for each colour for 4 peaks are indicated.

Figure 18 shows that the highest mass absorption coefficient between blue, deep red and amber is for amber light, which was expected. However this difference is not statistically significant (P>0.1) and as a results these results are not so reliable. Amber light is not strongly absorbed by the algae and they behave like they are under low light intensity. Thus microalgae have to increase their pigmentation, in order to absorb more light, which results in higher mass absorption coefficient. Deep red and blue cultures have lower mass absorption coefficient because these colours are highly absorbed by microalgae. Microalgae sense these colours as high light intensities and adjust their pigmentation by decreasing their pigment content. As a result they have lower mass absorption coefficient. Guus de Vries (2014) conducted the same measurements and their results are depicted in figure 19.

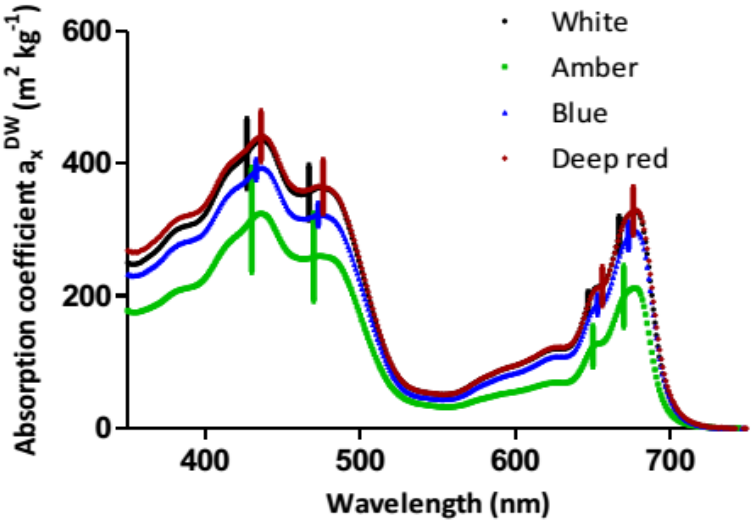

Figure 19: Mass absorption coefficient for five different colours used to grow C. reinhardtii. Results for different experiment were averaged for each colour. The standard deviation for all the colours is shown at each wavelength, which result in a black cloud of standard deviations. These data from experiments conducted by Guus de Vries.

According to figure 19, amber light gives significant lower results, compare to the other colours, which is not expected as it was explained above. For the same reason cultures under blue or red light expected to have lower mass absorption coefficient, because these lights are strongly absorbed and microalgae think that they are under high light intensity. As a result they have to decrease their pigmentation.

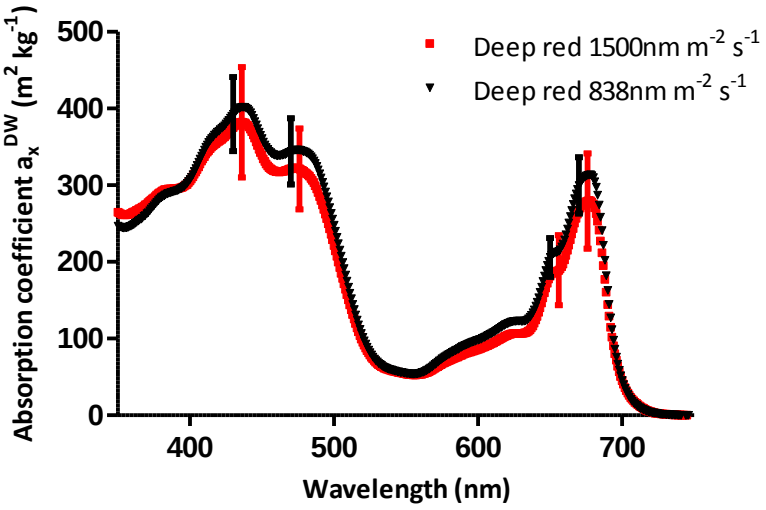

Figure 20: Average mass absorption coefficient of at least 5 measurements for deep red in 1500 µmol m^{-2} s^{-1} and 10 measurements for deep red in 835 µmol m^{-2} s^{-1}. Standard deviation for 4 different wavelengths are indicated.

Figure 20 shows that in deep red cultures when the light intensity is lower the mass absorption coefficient seems to be higher. The explanation for this is that deep red is highly absorbed and when the light intensity is decreased the pigment content is decreased. On the other hand standard deviation are overlapping and their difference is not statistically significant (P>0.5).

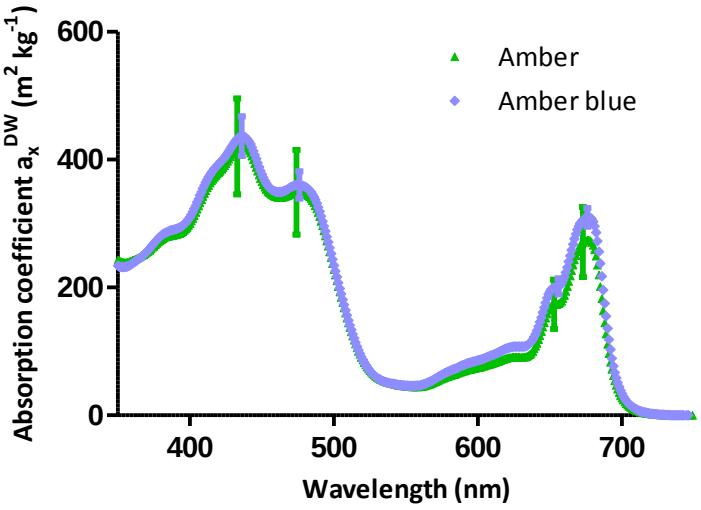

Figure 21: Average mass absorption coefficient of 10 measurements for amber and amber-blue light. Standard deviation for 4 different wavelengths are indicated.

Figure 21 illustrates that absorption coefficient for amber blue doesn't differ from the amber one (P>0.5). It was expected that because blue light is better absorbed by microalgae and they don't have to produce as many pigments as for the amber light, the absorption coefficient for amber-blue light

would be slightly higher. The standard deviations for the plots are overlapping so we cannot conclude to a clear finding.

Maximum biomass yield on light measurements

Experiments in chemostat mode were conducted in order to estimate the yield of biomass on light ($Y_{x/ph}$ g mmol^{-1}) and the maintenance requirement (r_m mmol g^{-1} h^{-1}). The experiments as it was already mentioned were performed under low light intensities, because in these intensities photosystems work at maximum efficiency. The dilution rates were ranging from 0.013 h^{-1} to 0.053 h^{-1} for white light and 0.015 h^{-1} and 0.029h^{-1} for blue light. For each dilution rate biomass density C_x (g L^{-1}) and photon flux density absorbed PFD_{abs} (µmol m^{-2} s^{-1}) were measured. With these measurements the specific photon consumption rate q_{ph} (mmol g^{-1} s^{-1}) could be calculated using equation 6. Table 3 and 4 illustrate biomass density, photon flux density and specific photon consumption rate for different growth rates for white and blue light respectively.

Table 3: Biomass density, absorbed photon flux density and specific photon consumption rate for different dilution rates for white light. Standard deviations for each value are included in the table.

Growth rate µ(h^{-1})	Biomass density C_x (g L^{-1})	Photon flux density absorbed PFD_{abs}(µmol m^{-2} s^{-1})	Specific photon consumption rate q_{ph} (mmol g^{-1} s^{-1})
0.013±0.001	1.02±0.2	80	20.1±5
0.028±0.001	0.46±0.2	73	41.2±5.5
0.039±0.001	0.42±0.1	65	40.1±9.4
0.053±0.001	0.33±0.08	60	46.7±4.9

Table 4: Biomass density, absorbed photon flux density and specific photon consumption rate for different dilution rates for blue light. Standard deviations for each value are included in the table.

Growth rate µ(h^{-1})	Biomass density C_x (g L^{-1})	Photon flux density absorbed PFD_{abs}(µmol m^{-2} s^{-1})	Specific photon consumption rate q_{ph} (mmol g^{-1} s^{-1})
0.015±0.001	0.18±0.2	25	36.2±4.4
0.029±0.001	0.13±0.2	22	45.9±2

In a graph of q_{ph} versus µ the intercept of the trend line is the maintenance requirement and the slope can help to calculate the biomass yield on light according to the equation 9:

$$q_{ph} = \frac{\mu}{Y_{x/ph}} + r_m \qquad (9)$$

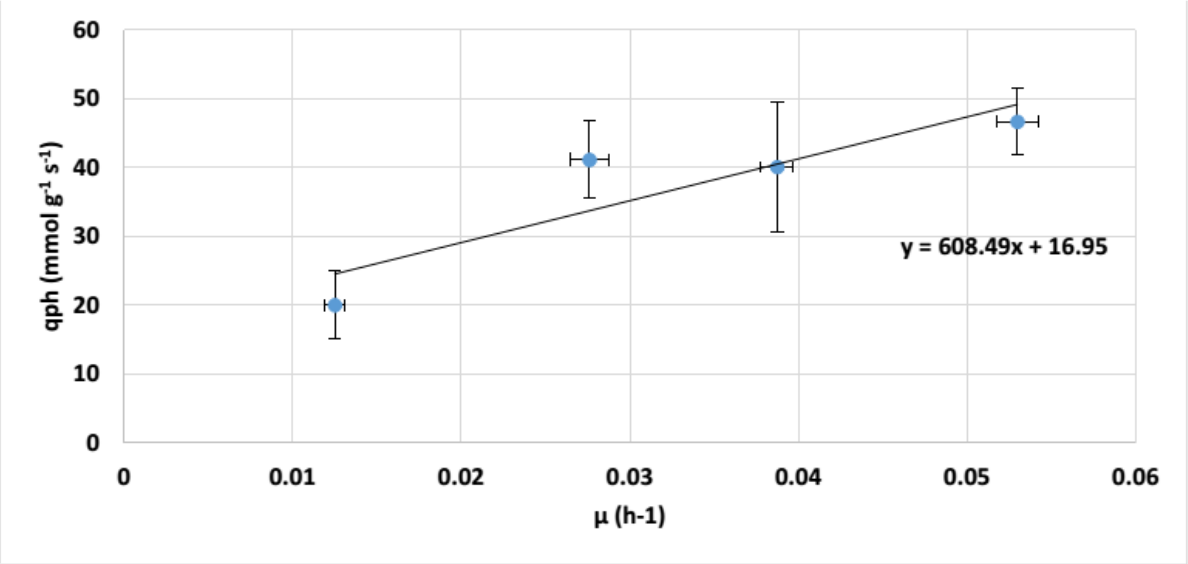

Figure 22: Specific photon consumption rate versus growth rate under white light.

Figure 22 illustrates the specific photon consumption rate versus growth rate for white light. The inverse of the slope gives $Y_{x/ph}$ = 1.64 g mol^{-1} and the offset of the line gives r_m = 17 mmol g^{-1} h^{-1} with a R^2 of 0.8. In chemostat experiments the dilution rate is steady but the biomass content differed day by day. Moreover growth of microalgae on the glass of the reactor was observed. This can affect the accuracy of outgoing light sensor and in low light intensities this effect becomes stronger. This biomass yield is higher than biomass yield for high light intensities, because at high light intensities the antenna complexes become saturated and the photoinhibition appears.

According to literature this biomass yield is too high. Takache et al. (2010) estimated the observed $Y_{x/ph}$ for *Chlamydomonas reinhardtii* at 110, 500 and 1000 μmol m^{-2} s^{-1} as 1.11, 0.73 and 0.51 g mol^{-1}. This is the observed biomass yield on light and it is not corrected for maintenance requirements. This literature verifies that in low light intensities the $Y_{x/ph}$ is higher and that in high light intensities photosystems don't work so efficiently. It can be seen comparing the results of biomass yield under white light at high light intensity (Figure 19) and at low light intensities (Figure 22). Under high light intensities the biomass yield was almost 0.4 g mol^{-1} when under low light it was 1.64 g mol^{-1}. Kliphuis et al. (2012) conducted the same experiment, with *Chlamydomonas reinhardtii* under red light at intensities <100 μmol m^{-2} s^{-1} and investigated biomass yield on light equal to 1.25 g mol^{-1}.

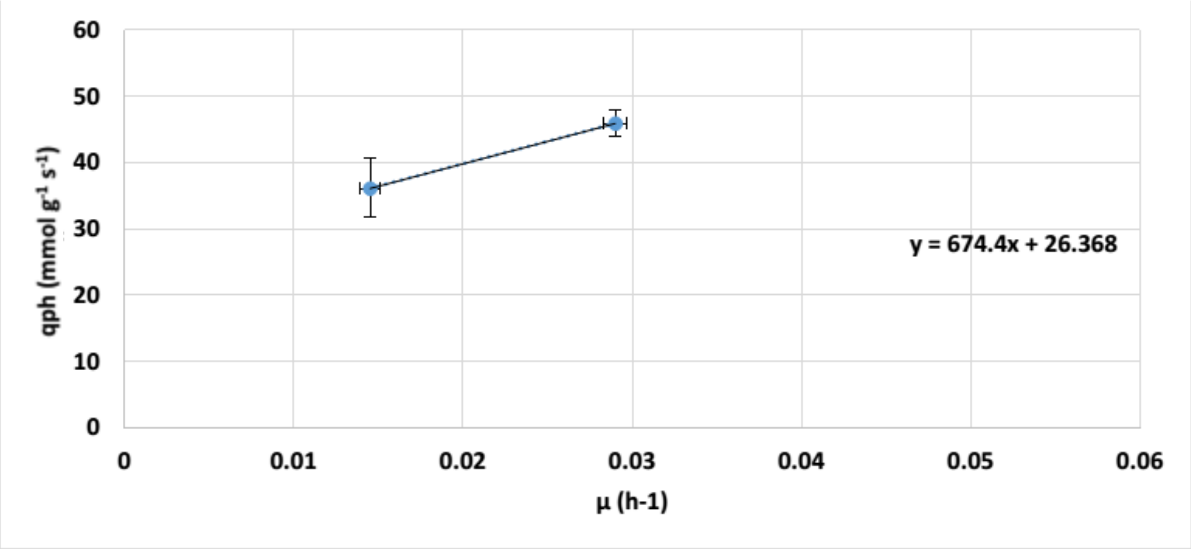

Figure 23: Specific photon consumption rate versus growth rate under blue light

Figure 23 illustrates the specific photon consumption rate versus growth rate for blue light. The inverse of the slope is gives $Y_{x/ph}$ =1.48 g mol^{-1} and the offset of the line gives r_m =26.37 mmol g^{-1} h^{-1}. For the blue light there are only 2 dilution rates due to limited time for experiments so the trend line is not reliable. More points of dilution rates are needed in order to create a more reliable trendline and equation.

Conclusion

This report investigated the influence of different light colours at high light intensities on the volumetric productivity of *Chlamydomonas reinhardtii*. The results illustrated that all light coulours, except the combination of amber-blue, showed lower volumetric productivity compared to white light. Our hypothesis was that higher light absorption per cell would result in lower volumetric productivity, while lower light absorption per cell result in higher productivity compared to the white light. The obtained data shows very clearly that blue and red light, which are better absorbed light colours than white, result in lower volumetric productivity. Moreover, the results indicates that despite the amber light shows reduced productivity, the addition of a small amount of blue light results in higher volumetric productivity than in white light. One possible explanation is that blue light possibly activates a mechanism and makes them fully functional. It possibly activates something in the algae which renders them fully functional. Unfortunately there are not much literature and it is not known clearly what the influence of blue light in the metabolism of microalgae is. The increased volumetric productivity of amber-blue light seems to verify our hypothesis, because penetrates deeper in a culture and it is not so well absorbed by microalgae so we expected higher productivity.

For the biomass yield on light, the amber with a small addition of blue light higher biomass yield (0.76 g mol^{-1}) compare to white light (0.7 g mol^{-1}). Moreover the biomass yield on light for chemostat experiments under low light intensities showed higher results, 1.64 g mol^{-1} for white and 1.48 g mol^{-1} for blue light, than under high light intensities, 0.39 g mol^{-1} and 0.2 g mol^{-1} respectively. This was expected because in high light intensities the antenna complexes in the algal photosystem becomes saturated.

As for the mass absorption coefficient the difference between all colours was not clear but the highest mass absorption coefficient seems to be obtained for cultures under amber light. This makes sense, because amber light is not absorbed so well, thus microalgae have to increase their pigmentation in order to absorb more light. The lowest mass absorption coefficient was investigated for blue light, because this is easiest absorbed by microalgae, thus they have to reduce their pigmentation in order not to become oversaturated.

The quantum yield measurement showed that for the cultures grown under deep red, red, amber and amber-blue light their photosystems were not operating at maximum capacity, possibly as a result of high light damage. However for cultures under white or blue light their photosystem II is more active.

The chemostat experiments showed that white light the biomass yield was 1.64 g mol^{-1} and maintenance was 16.95 mmol g^{-1} h^{-1}. For blue light experiments only for two dilution rate were performed, were the biomass yield was 1.48 g mol^{-1} and the maintenance was 26.37 mmol g^{-1} h^{-1}.

Recommendations for further research

The results were more or less as it was expected. They verified that when the light absorption rate per cell is high the volumetric productivity will be low, while the volumetric productivity will be higher when the light absorption rate per cell is low. However the results for the amber light were not as we expected. The volumetric productivity was lower than the predicted one and the culture was unstable. One explanation could be the antifoam solution that was added in the reactor. Maybe it was too much and influenced the culture, making it unstable. The amount of antifoam was 10-20 ml per day and not precise or constant. The dosage of antifoam should be checked and investigated in order to be harmless for the culture.

Moreover, the absorption spectrum seems and has to be different for amber, blue and red light, because when a light is not well absorbed, such amber light, the microorganism has to increase its pigmentation and has higher absorption coefficient. However the standard deviations of each spectrum are overlapping and the results are not clear. Thus a better investigation of the pigment content will give more insight knowledge of what happens in microalgae pigmentation and metabolism when they are exposed in a well on not absorbed monochromatic light.

In addition, in chemostat experiments under low light intensities the measurement for dry weight were not stable and that is depicted in the error bars of the graphs (Figure 22, 23). This happened because there was growth of cells on the glass of the reactor and a big amount of biomass were wasted there. An option to avoid this glass growth of algae is to improve the mixing of the culture in the reactor. This can be done by increasing the airflow in the reactor.

The next step is to use this research as a proof of principle to stimulate antenna mutants. By using the promising technology of antenna mutants, experiments can be conducted to investigate if microalgae with truncated antenna complexes results higher volumetric productivity. In principle they absorb less incoming light intensity, light penetrates deeper and more algae can be produced under the same light intensity when they are mutants.

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Appendix

Medium recipe

The macronutrients were all prepared in stock solutions and concentrated. For the Hütner solution preparation the first two salt were dissolved in 400mL of demin water and the pH was adjusted with 4M KOH (or NaOH) to about 5.5. The remaining salts were dissolved in the order indicated in 400ml of demin water and until one by one all salt were completely dissolved. After that the two solutions were mixed and pH was readjusted to around 6.7 with 4M KOH (or NaOH). Finally the mixed solution was filled up to 1L and stored in dark at 4-8 °C.

Table 5: Medium composition

Medium		**Trace Element**	
Macronutrients	**final concentration (g L^{-1})**	**Micronutrients (Hütner)**	**final concentration (g L^{-1})**
KH_2PO_4	0.706	$FeSO_4 \times 7H_2O$	0.01
K_2HPO_4	1.465	$Na_2EDTA \times 2H_2O$	0.1107
$MgSO_4.7H_2O$	0.560	$ZnSO_4.7H_2O$	4.40E-02
$CaCl_2.2H_2O$	0.114	H_3BO_3	2.28E-02
$CO(NH_2)_2$	0.99	$MnCl_2.4H_2O$	1.01E-02
		$CoCl_2 \times 6H_2O$	3.22E-03
		$CuSO_4.5H_2O$	3.14E-03
		$(NH_4)_6Mo_7O_{24} \times 4H_2O$	2.20E-03

Productivity Model

A productivity model was used in order to predict the productivity, dry weight and dilution rate for different light colour. The model calculated these values according to the following parameters (Table 5 and 6).

Table 6: Photobioreactor parameters

Constant	**Symbol**	**Value**
Incoming light intensity (mol m^{-2} s^{-1})	$I_{ph,PAR}(0)$	1.50 10^{-3}
Depth of the reactor (m)	d	0.014
Irradiated surface per reactor volume (m^2 m^{-3})	A/V	71.43

Table 7: Chlamydomonas reinhardtii parameters

Constant	**Symbol**	**Value**
Molecular weight biomass (g $Cmol^{-1}$)	M_x	24
Max. CO_2 production rate (mol_{CO2} $Cmol^{-1}$ s^{-1})	$q^c_{CO2,m}$	7.50 10^{-5}
Max. yield of CO_2 fixation on photons (mol_{CO2} mol_{ph}^{-1})	$Y^c_{CO2/ph,m}$	0.10
Yield of sugars on CO_2 (mol_{CH2O} mol_{CO2}^{-1})	$Y_{CH2O/CO2}$	1.00
Maintenance coefficient (mol_{CH2O} $Cmol^{-1}$ s^{-1})	m_{CH2O}	3.50 10^{-6}
Biomass yield on sugars (Cmol mol_{CH2O}^{-1})	$Y_{x/CH2O}$	0.59
Dimensionless d-enhancement (-)	d	1.75

The light intensity is not the same in the whole reactor path, because it decreases while light penetrates deeper in the reactor. According to this, specific growth rate is not constant as well and an average value has to be calculated. This can be done by dividing the reactor depth z in 100 layers, with Δz as length for each layer, and calculate an average specific growth rate $\mu_{PI(z-½Dz)}$, which is the sum of depth specific growth rates. This calculation is done using the following equations:

$$\mu_{PI\left(z-\frac{z}{2}\right)} = (q^{C}_{CO2}\left(z-\frac{z}{2}\right) - m_{CH2O}) \times y_{X/CH2O} \quad (10)$$

$$q^{C}_{CO2}\left(z-\frac{z}{2}\right) = Q^{C}_{CO2,m} \times \tanh \frac{y^{C}_{\frac{CO2}{ph},m} \times q_{ph(z-\frac{z}{2})}}{q^{C}_{CO2,m}} \quad (11)$$

$$q_{ph(z-\frac{z}{2})} = \frac{I_{ph,par(0)} - I_{ph,par(z)}}{C_X \times \Delta z} \quad (12)$$

$$I_{ph,par(z)} = I_{ph,par(0)} \times e^{-a_{x,par} \times C_x \times z \times d} \quad (13)$$

Using equation 13 the light intensity at depth z can be calculated, while equation 12 can calculate the specific light absorption rate at each layer. The superscript c in equation 11 refers to chloroplast. Using this equation the rate of photosynthesis at each layer can be calculated, while equation 10 calculates the specific growth rate. Each wavelength have a different mass absorption coefficient ax. Due to this there are differences in each wavelength dependent mass absorption coefficient (ax,λ*En,PAR), the sum of which is the absorption coefficient. Moreover the biomass concentration is missing in order to calculate the volumetric productivity. Using Microsoft Excel the optimal biomass concentration can be found with the specific growth rate. This is the method to predict the volumetric productivities that were shown in Material and methods section.

Light intensity measurements

The incoming light intensity was measured on the irradiated area of the photobioreactor at 28 points. The reactor and the LED lights were placed in a proper position in order to have the best light distribution over the surface of the reactor. The following tables indicate the ingoing and outgoing light intensity for each colour at these 28 points, but for the calculations only the average incoming light was used. For the outgoing light intensity 27 points were measured because one point was captured by the turbidity sensor of the reactor.

Table 8: Ingoing and outgoing light intensity for photobioreactor under blue light

Blue light									
Ingoing light intensity	1442	1493	1372	1386	Outgoing light intensity	8	9	9	7
	1488	1361	1492	1685		9	11	11	12
	1656	1618	1600	1592		10	11	12	11
	1459	1579	1573	1656		10	11	12	-
	1474	1488	1548	1270		10	11	12	10
	1397	1547	1413	1435		9	10	10	9
	1488	1317	1498	1379		7	9	8	7
			Average	**1489**				**Average**	**10**

Table 9: Ingoing and outgoing light intensity for photobioreactor under deep red light at high intensity

Red light (High intensity)									
Ingoing light intensity	1303	1310	1251	1230	Outgoing light intensity	10	11	11	10
	1604	1590	1619	1599		13	13	13	12
	1620	1602	1686	1573		12	13	13	12
	1614	1700	1681	1674		12	13	13	-
	1631	1648	1628	1203		12	13	13	10
	1542	1592	1608	1499		11	12	11	10
	1232	1289	1302	1269		8	8	9	8
			Average	**1503**				**Average**	**11**

Table 10: Ingoing and outgoing light intensity for photobioreactor under deep red light at lower intensity

Red light (Lower intensity)									
Ingoing light intensity	737	754	748	708	Outgoing light intensity	10	11	11	10
	863	855	870	860		13	13	13	12
	880	862	905	845		12	13	13	12
	875	930	901	898		12	13	13	-
	897	905	882	745		12	13	13	10
	850	863	863	845		11	12	11	10
	727	795	796	753		8	8	9	8
			Average	**836**				**Average**	**11**

Table 11: Ingoing and outgoing light intensity for photobioreactor under deep red light at lower intensity (2nd run of experiments)

Red light (Lower intensity)									
Ingoing light intensity	740	756	751	708	Outgoing light intensity	11	11	11	10
	865	860	874	862		13	13	13	12
	885	867	909	848		12	13	13	12
	880	937	907	901		12	13	13	-
	900	909	887	745		12	13	13	10
	850	863	863	845		11	12	11	10
	729	795	796	753		8	8	8	8
			Average	**838**				**Average**	**11**

Table 12: Ingoing and outgoing light intensity for photobioreactor under amber light

Amber light									
Ingoing light intensity	1389	1469	1358	1380	Outgoing light intensity	10	11	11	10
	1521	1614	1521	1524		13	13	13	12
	1593	1604	1532	1493		12	12	13	12
	1650	1613	1606	1487		12	13	13	-
	1628	1565	1498	1320		12	13	13	10
	1582	1533	1486	1494		11	12	11	10
	1367	1409	1357	1357		8	8	9	8
			Average	**1504**				**Average**	**11**

For the chemostat experiments low light intensities were used for white and blue light. The outgoing light was changing because the dilution rate was changing as well.

Table 13: Ingoing light intensity for photobioreactor under white and blue light used for the chemostat experiments

	White light				Blue light			
Ingoing light intensity	79	81	81	80	30	29	29	28
	79	83	82	80	30	30	30	29
	80	84	84	81	31	32	32	30
	81	86	85	82	31	33	33	31
	80	85	85	82	29	32	32	28
	79	84	83	81	26	31	30	28
	79	82	82	81	24	26	27	29
			Average	**81**			**Average**	**30**

Volumetric productivity, biomass concentration and dilution rate data

Daily measurements for productivity, dry weight and dilution rate were conducted for cultures under different light colours, when they were in steady state. The following graphs illustrate these measurements. The length of experiments was not the same for all because some cultures flocculated and died before others.

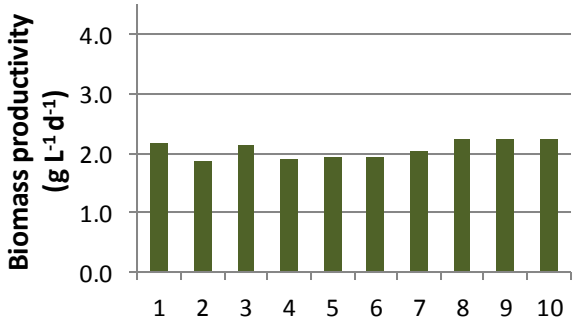

Figure 25: Biomass productivity for Chlamydomonas reinhardtii under blue light.

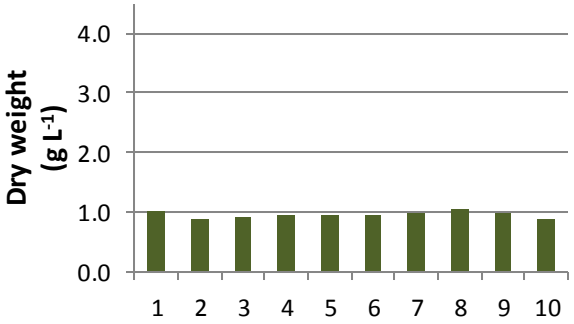

Figure 24: Dry weight biomass composition for Chlamydomonas reinhardtii under blue light

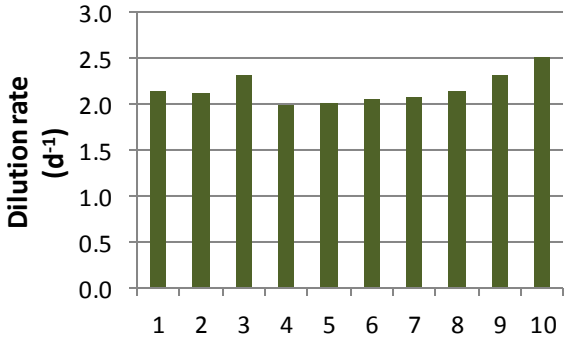

Figure 27: Dilution rate for Chlamydomonas reinhardtii under blue light

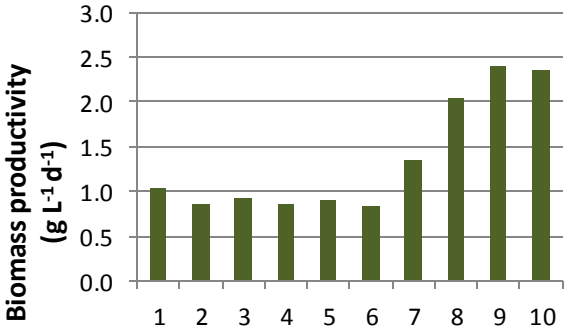

Figure 26: Biomass productivity for Chlamydomonas reinhardtii under deep red light 1500 µmol m^{-2} s^{-1}.

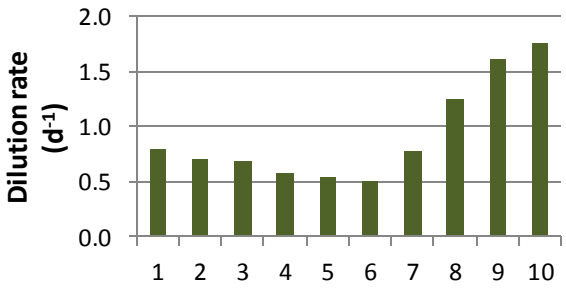

Figure28: Dilution rate for Chlamydomonas reinhardtii under deep red light at 1500 µmol m^{-2} s^{-1}.

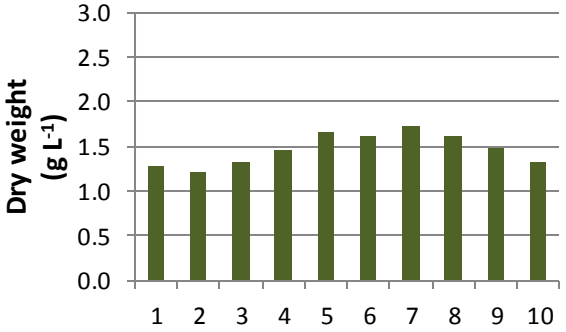

Figure29: Dry weight biomass concentration for Chlamydomonas reinhardtii under deep red light at 1500 µmol m^{-2} s^{-1}.

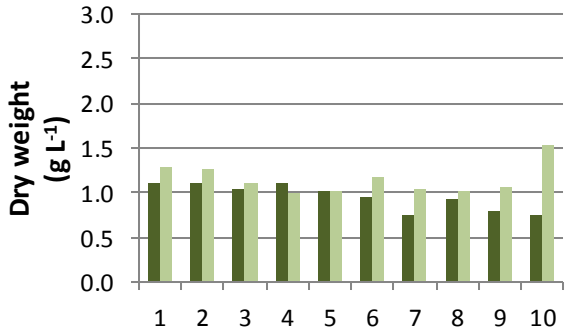

Figure 30: Dry weight biomass concentration for Chlamydomonas reinhardtii under deep red light at 838 µmol m^{-2} s^{-1}. Green and light green bars represent two different runs of same experiment.

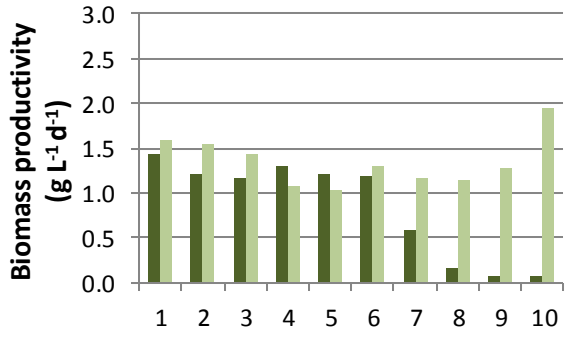

Figure31: Biomass productivity for Chlamydomonas reinhardtii under deep red light at 838 µmol m^{-2} s^{-1}. Green and light green bars represent two different runs of same experiment.

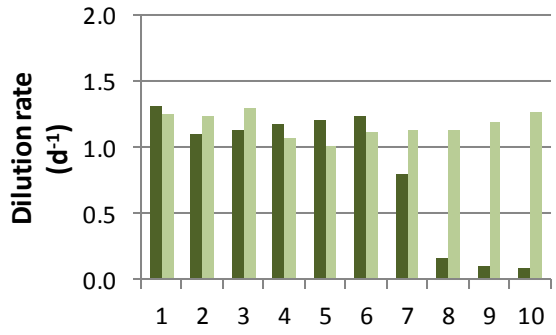

Figure 32: Dilution rate for Chlamydomonas reinhardtii under deep red light at 838 µmol m^{-2} s^{-1}. Green and light green bars represent two different runs of same experiment.

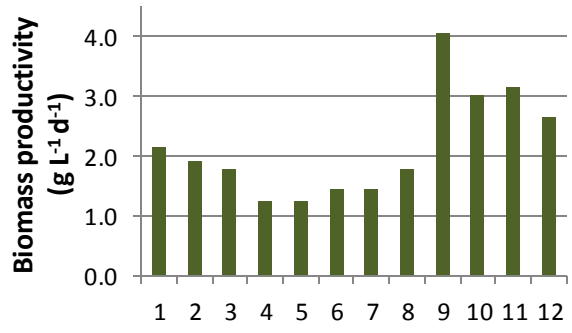

Figure 33: Biomass productivity for Chlamydomonas reinhardtii under amber light at 1500 µmol m^{-2} s^{-1}.

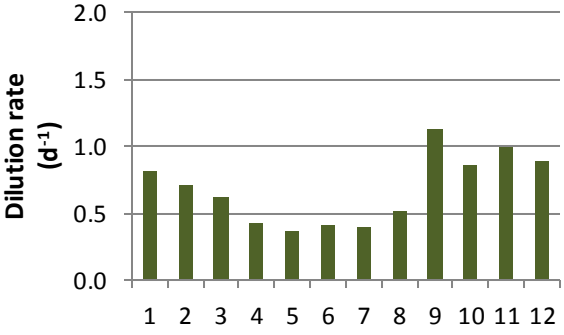

Figure 34: Dilution rate for Chlamydomonas reinhardtii under amber light 1500 µmol m^{-2} s^{-1}.

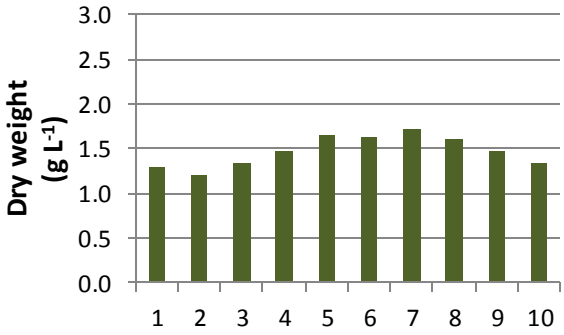

Figure 35: Dry weight biomass concentration for Chlamydomonas reinhardtii under deep red light at 1500 µmol m^{-2} s^{-1}.

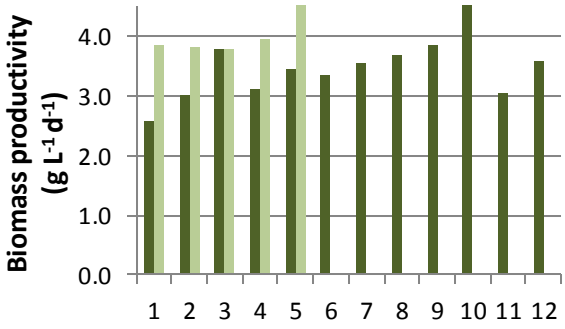

Figure 36: Biomass productivity for Chlamydomonas reinhardtii under 1436 µmol m^{-2} s^{-1} of amber and 51 µmol m^{-2} s^{-1} of blue light. Green and light green bars represent two different runs of same experiment.

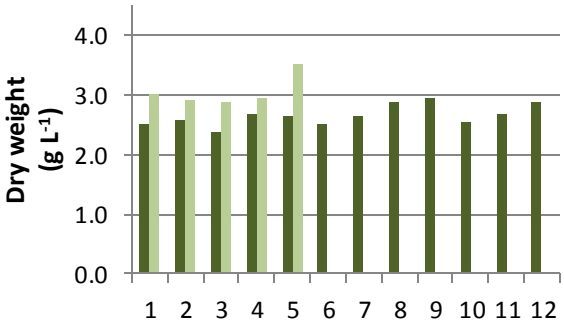

Figure 37: Dry weight biomass concentration for Chlamydomonas reinhardtii under 1436 µmol m^{-2} s^{-1} of amber and 51 µmol m^{-2} s^{-1} of blue light. Green and light green bars represent two different runs of same experiment.

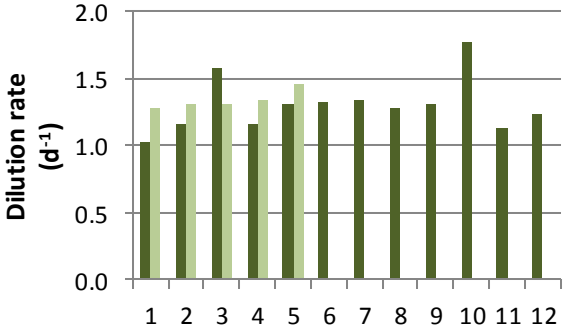

Figure 38: Dilution rate for Chlamydomonas reinhardtii under 1436 µmol m^{-2} s^{-1} of amber and 51 µmol m^{-2} s^{-1} of blue light. Green and light green bars represent two different runs of same experiment.