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Effects of different light intensities, light spectra and temperatures on the growth and fatty acid composition of the microalgae *Acutodesmus obliquus*

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Aquaculture

1 Content

Content

Abstract

The subject of this thesis were the effects of cultivation of different light intensities, spectral light areas and temperatures on growth and fatty acid composition in the microalgae *Acutodesmus obliquus*. While previous experiments mainly used light emitting diodes and looked at light effects of narrow line spectra, filter foils were used in this study to analyse the differences in growth and fatty acid profiles caused by spectral areas of visual blue, green and red light. Cultivation temperatures between 20 °C and 35 °C were tested with light intensities between 120 µmol m^{-2} s^{-1} and 800 µmol m^{-2} s^{-1}. The established opinion, that the usage of green light for cultivation of plants leads to low biomass growth, was disproved in the case of *A. obliquus*, which had high growth values under green light cultivation. Growth was always best in the fill solar spectrum control group, followed by the red light group. With increasing biomass concentrations, the differences between the biomass values for the red and green light group became less significant and the green light group outperformed the red light group during high light intensity. Cultivation under blue light always gave the lowest growth values. With increasing light intensities, growth increased. The best biomass results were achieved for cultivation at 30 °C under 800 µmol m^{-2} s^{-1} in the green light and full solar spectrum control group. Lipids of the algae were extracted, and fatty acids were subsequently analysed by GC-MS. The main fatty acids of *A. obliquus* were the saturated fatty acid 16:0 with around 44 % and the polyunsaturated fatty acid 18:3 with around 21 %. With increasing temperatures, overall saturation in the fatty acid profile of *A. obliquus* increased and this effect was highest when using red spectral light. A cultivation temperature of 20 °C gave the most favourable fatty acid composition, meaning a high amount of polyunsaturated and less saturated fatty acids. However, growth under these conditions was low. The second best fatty acid composition was achieved in the experiment using high intensities, which also gave the best growth results,

especially for green and full solar light. This combination is therefore recommended if the aim is the commercial production of polyunsaturated fatty acids from *A. obliquus*.

Table of abbreviations

~	around/roughly
A. obliquus	*Acutodesmus obliquus*
amu	atomic mass unit
ANOVA	analysis of variance
ATP	adenosine triphosphate
CO_2	carbon dioxide
DW	dry weight
FA	fatty acid
g L^{-1}	gram per litre
GC-MS	Gas chromatography – mass spectrometry
MUFA	monounsaturated fatty acid
NADP	nicotinamide adenine dinucleotide phosphate
OD	optical density
OD-DW	correlation of optical density to dry weight
PAM	Normally ‘pulse amplitude modulation’, in this work used as abbreviation for ‘pulse amplitude modulation chlorophyll fluorometer’
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
TAG	triacylglycerols
Y(II)	effective PSII quantum yield
µmol m^{-2} s^{-1}	micromole per square meter second

2 Introduction

Worldwide, algae account for more than half of the primary production at the base of the food chain (Van den Hoek, 1998). Microalgae are unicellular, photosynthetic aquatic organisms that show high productivity rates (Chisti, 2007; Day et al., 1999; Suganya et al., 2016). The main interest was the production of biofuels, which started cultivation experiments and lead to the first large-scale cultivation in the early 1960s of *Chlorella* spp. in Japan (Mata et al., 2010; Spolaore et al., 2006). However, microalgae can also be a valuable source for the production of secondary metabolites and they offer unique possibilities for uses in different areas, such as wastewater treatment, bio fixation of CO_2, production of chemicals and bulk products and utilisation areas like nutrition, cosmetics and pharmaceuticals (Mata et al., 2010; Suganya et al., 2016). Recently microalgae have gained more attention as potential sources of fatty acids (FAs) for human food and animal feed supplementation (Doughman et al., 2007). Replacing saturated fatty acids (SFAs) in the diet with polyunsaturated fatty acids (PUFAs), has been shown to have multiple benefits on human health, especially the PUFAs are characterized by a long chain and omega-3 unsaturation (Riediger et al., 2009; Ruxton et al., 2004). The essential fatty acids eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) need to be taken up nutritionally, however the main source for humans is still fish and fish oil (Tacon & Metian, 2009). This however poses the problem of dietary restrictions like allergies or a vegetarian lifestyle, which could be overcome by producing essential FAs or their precursors from microalgae (Doughman et al., 2007; Riediger et al., 2009). Another important area is animal feed, which requires certain amount of PUFAs to ensure animal health and welfare, but also to achieve adequate amounts of PUFAs in meat, eggs and milk to satisfy consumer demands (Chee et al., 2005; Davis & Kris-Etherton,

2003; Rymer & Givens, 2005). The characteristics of microalgae to synthesise and accumulate great amounts of PUFAs are making them one of the most important feed sources in aquaculture, either as direct source for herbivorous fish or as addition replacing fish oil in feed for carnivorous fish, as fish oil is becoming a limited resource due to an increasing demand (Patil et al., 2005; Tacon & Metian, 2009). To produce microalgae on a large scale, open ponds or photobioreactors can be used and a lot of research has gone into optimizing productivity for different, economically interesting species (Mata et al., 2010; Patil et al., 2005; Wang et al., 2014). However, to have an optimum cost effectiveness of production, it is necessary to maximise productivity and efficiency of photobioreactors with microalgae cultures (Stephens et al., 2010). This would lead to high profitability, but microalgae cultivation operation units with simultaneously high productivity and high efficiency are rarely achieved (Goldman, 1979). Therefore, research is needed to find ways to maximise productivity by finding influences which stimulate growth and/or production of secondary metabolites. In terms of manipulating the composition of FAs in microalgae, studies showed an influence of temperature, light intensities and wavelengths used for cultivation (Hultberg et al., 2014a; Ooms et al., 2017; Xin et al., 2011). However, studies have mostly been conducted using light emitting diodes, which give narrow line spectra (de Mooij et al., 2016; Hultberg et al., 2014a; Ooms et al., 2017). Using the sun as light source for microalgae in photobioreactors decreases energy costs, however here light spectra can only be controlled for by using filters, which give a broader spectral light area than light emitting diodes. The microalgae *Acutodesmus obliquus* has been deemed a suitable candidate for large scale production due to its high productivity and ability to grow well in cheap media (Abomohra et al., 2013; Breuer et al., 2012; Hindersin, 2013). However, little is known about the growth and fatty acid composition of *A. obliquus* under different spectral light areas combined with different

temperatures and light intensities. This thesis is the first systematic approach to gain more knowledge in this area, addressing the hypotheses that:

H1: Algae growth and metabolism are directly correlated with light and temperature, and

H2: Changes in light and temperature can be used for a targeted production of fatty acids.

3 Theoretical background

3.1 Fatty acids

3.1.1 General

Fatty acids (FAs) are carboxylic acids with an aliphatic chain of variable length, their usual form of presence is as triacylglycerols or phospholipids in membranes and storage lipids (Heldt & Piechulla, 2010). In nature, they typically have an even number of carbon atoms and the length of the chain usually lies between 16 and 26 carbons (Quehenberger et al., 2011; Ruxton et al., 2004). FAs that exclusively have single bonds between adjacent carbon atoms are called ‘saturated’, if they possess one or more double bonds between carbon atoms, they are termed ‘unsaturated’. The double bonds are usually present in *cis* configuration and interrupted by at least one methylene group, although numerous exceptions exist (Christie, 1998). With increasing unsaturation, the melting point of the fatty acid decreases (Ruxton et al., 2004).

The nomenclature is made up by the length of the hydrocarbon chain and the number of double bonds. If the FA possesses more than one double bond, it is called ‘polyunsaturated fatty acid’ (Ruxton et al., 2004). In the nomenclature, this is marked by an n-x, where x is the position of the first double bond when counting is started at the methyl group end of the hydrocarbon chain (Ruxton et al., 2004). DHA for example has a chain length of 22 carbons and six double bonds, from which the first one lies between the third and fourth carbon. This means that DHA has the name 22:6(n-3) in fatty acid nomenclature.

3.1.2 Omega-n-fatty acids

Especially n-3 and n-6 fatty acids have been subject to comprehensive research and today, knowledge about the positive impact of n-3 fatty acids on human and animal

health is well established (Riediger et al., 2009; Ruxton et al., 2004). The long chained n-3 fatty acids play a significant role during infant development and seem to have protecting properties against cancer, cardiovascular diseases and numerous mental illnesses like dementia, depression and attention-deficit hyperactivity disorder (Riediger et al., 2009; Ruxton et al., 2004). The focus lies especially on replacing saturated fats with PUFAs, which is widely accepted by the public as part of a healthy lifestyle (Gogus & Smith, 2010). n-6 fatty acids like arachidonic acid (20:4, ARA) and its precursor linoleic acid (18:2, LA) are usually present in higher concentrations than n-3 fatty acids. These FAs can pose some problems, as they are precursors for eicosanoids with inflammatory properties and are competing with n-3 fatty acids for conversion enzymes (Ruxton et al., 2004; Simopoulos, 1999). Eicosanoids are signalling molecules involved in the regulation of several processes in the body, both n-3 and n-6 fatty acids are precursors of them (Lands, 1992). The major factor influencing the outcome of enzymatic pathways seems to be the ratio of n-3 to n-6 fatty acids (Riediger et al., 2009). It needs to be noted that although high concentrations of n-6 fatty acids in relation to n-3 fatty acids may be harmful to human and animal health, they are still serving important functions in the body (Lands, 1992).

It appears that the beneficial health effects of n-3 fatty acids are associated with their incorporation in the membrane phospholipids (Clandinin et al., 1994). The major dietary n-3 fatty acids are α-linolenic acid (18:3, ALA), EPA (20:5) and the previously mentioned DHA (22:6). EPA and DHA can be synthesised from the precursor ALA, but efficiency of this transformation seems to vary and is generally said to be low (Plourde & Cunnane, 2007). Recent studies show that the transformation efficiency of ALA to EPA and DHA is influenced by diet and sex and increases with a lower dietary intake of EPA and DHA, e.g. during a vegetarian lifestyle (Welch et al., 2010). A study

by Sanders and Roshanai (1983) showed that even though dietary supplementation of ALA with linseed oil increased platelet EPA to a lesser amount than supplementation with fish oil containing EPA and DHA, the increase was still of a moderate level. The current main source of EPA and DHA is fish oil, which is becoming a very limited resource due to insufficient availability to meet the growing demand (Tacon & Metian, 2009). Additionally, the lipid fraction of some fish species is showing high levels of environmental pollutants like dioxins and heavy metals, especially mercury (Riediger et al., 2009; Ruxton et al., 2004). Furthermore, fish oil supplements can cause nausea and pose the problem of dietary restrictions, e.g. for vegetarians or people allergic to fish (Riediger et al., 2009). A dietary supplementation of ALA leads to a higher ratio of n-3 to n-6 fatty acids, and therefore the conversion ratio of ALA to EPA and DHA increases, as n-6 fatty acids have a lower chance to compete for desaturase and elongase enzymes (Budowski, 1988; Davis & Kris-Etherton, 2003). Microalgae have been proposed as one of the sources of dietary n-3 fatty acids to be used for direct human consumption or for enrichment of animal feed to increase EPA and DHA content in meat, eggs and milk (Chee et al., 2005; Davis & Kris-Etherton, 2003; Rymer & Givens, 2005).

3.1.3 Gas chromatography – mass spectrometry for analysis of fatty acids

Gas chromatography – mass spectrometry (GC-MS) is a method for the separation of components in a mixture by making use of their different boiling points. This happens in a specially coated column, which is heated by an oven in a defined temperature program (Sparkman et al., 2011). The substances are then forwarded into the MS part, where ionisation of the molecules by an electron shock leads to their fragmentation. This gives a characteristic fragmentation pattern for each molecule, also called 'chemical fingerprint', and together with the retention time from the GC, the molecule can be identified by comparison of the information to databases. A schematic illustration of a GC-MS can be seen in figure 1.

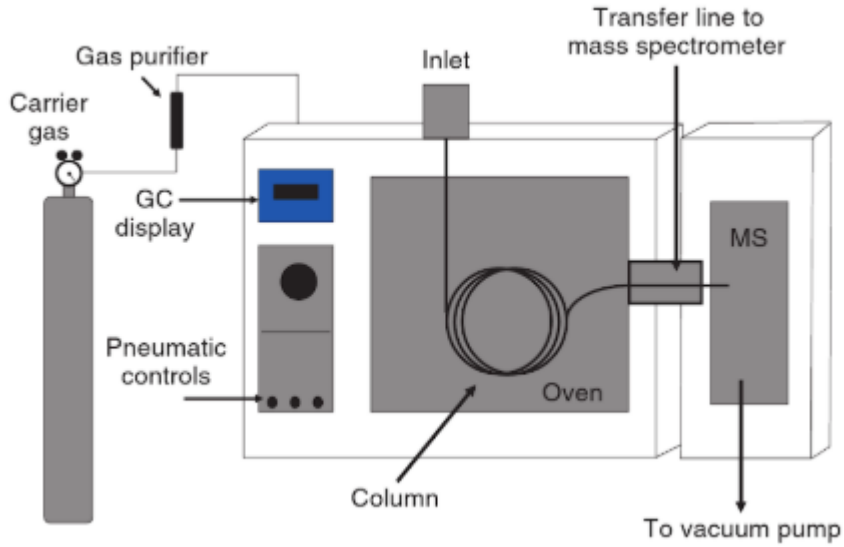

Figure 1: Schematic illustration of a typical GC-MS, from Sparkman et al. (2011). The sample is injected in the inlet and then transported by the carrier gas through the GC column and further into the MS.

The process starts when a sample is inserted into the heated inlet or injector via a syringe (Sparkman et al., 2011). Hydrogen or helium is then used as a carrier gas, often referred to as 'mobile phase', to forward the sample from the injector into a capillary column with an inner coating of a stationary phase. Due to the temperature differences between the heated inlet and the colder column, condensation of the sample onto the

column walls takes place. Their polarity and chemical structure cause the components to be selectively retarded by the stationary phase, but the elevated pressure in the GC, as well as gradually rising temperatures, lead to a successive release of them. The components subsequently pass a detector, where the change in electrical resistance gives a signal and the retention time of the molecule is determined. Still vaporised in the carrier gas and undamaged by the detector, they are transported to the MS part, where they are ionised. Here a vacuum is used to separate the ions according to their mass-to-charge ratio (*m/z*) and a detector produces an electrical signal for each fragment. As virtually all of the ions formed in GC-MS have a single charge, their *m/z* value is considered identical to the mass of the ion (Sparkman et al., 2011). The result of a GC-MS is a chromatogram containing differentiated peaks including information about their fragments and retention time. By comparing the chromatogram with a spectrum library, the components contained in the sample can be determined. If an internal standard has been added to the sample prior to analysis, the peak curves can be integrated and their area can be compared to that of the internal standard, which then gives a qualitative measurement of the components (Sparkman et al., 2011). One of the many possible applications of GC-MS is the structural analysis of fatty acids (Christie, 1998).

3.2 Photosynthetic organisms

3.2.1 Microalgae

Microalgae are microscopic, unicellular organisms that can exist individually or appear in groups or chains (Suganya et al., 2016). They are a phylogenetically diverse group, comprising eukaryotic protists, prokaryotic cyanobacteria and blue green algae (Day et al., 1999). In contrast to higher plants, they do not possess organs like roots or leaves, nevertheless the eukaryotic green algae are closely related to all land plants

(Karol et al., 2001). Like terrestrial plants, microalgae use photosynthesis to convert solar energy into chemical energy (ATP and NADP) as well as for the fixation of biomass (Day et al., 1999). Besides light that provides them with energy, they need a carbon source such as carbon dioxide for an autotrophic metabolism, a liquid medium to grow in and certain nutrients like phosphorus, nitrogen and other trace elements (Choi et al., 2015). Microalgae are present in both freshwater and marine water systems, where they are responsible for the production of approximately half of the atmospheric oxygen (Day et al., 1999). Due to their unicellular lifestyle, their generation time is much shorter than that of more complex plants and they complete their entire growth cycle within a few days (Choi et al., 2015; Mata et al., 2010). In addition to the productivity, microalgae cultivation poses the advantage of not requiring the usage of arable land (Chisti, 2007; Mata et al., 2010; Sheehan et al., 1998). Growing them on a large scale is therefore not in competition with food production, which makes their utilization for the synthesis of products like biofuel much more ethical than the usage of terrestrial crops (Suganya et al., 2016). Microalgae do not contain any lignin, a structural organic polymer which impedes efficient biofuel production from land plants (Milledge & Heaven, 2014) and their lipid to area ratio can be much higher than that of traditional crops for biodiesel (Chisti, 2007). All these facts make microalgae a promising non-food source of biofuels and other commercial applications (Suganya et al., 2016).

3.2.2 Active spectrum of photosynthesis

The first scientist to determine the active spectrum of photosynthesis was Theodor Wilhelm Engelmann, who published various microbiological studies concerning photosynthesis between 1881 and 1888 (Drews, 2005). One of his methods was growing microalgae together with aerotactic bacteria in a microscopic chamber,

proving that microalgae produce oxygen when they are exposed to light, because the oxygen-sensing bacteria accumulated in the illuminated area. By adding a prism to the microspectral apparatus, the light shining on the microalgae and bacteria was separated into different light spectra. Subsequently it was observed that the accumulation of bacteria started in the red region of the spectrum, continued to the orange region and went on from the blue to the violet region of the spectrum (Drews, 2005). Later on this was explained by the absorption maxima of the main pigment of photosynthesis, chlorophyll a, which have peaks at 430 and 680 nm (Brown, 1972). This led to the common interpretation that photosynthetic activity can only be high in the area of spectral maximum absorption of chlorophyll. The photosynthetic activity is supposedly reduced in the area of low absorbance, the 'green window' between 450 nm and 600 nm (Heldt & Piechulla, 2010). This opinion was reinforced when multiple studies with terrestrial plants found inhibited growth of plants under green light (Folta & Maruhnich, 2007; Muneer et al., 2014). However, the established opinion that green light cannot be efficiently used for photosynthesis has been criticised before, since various studies showed different outcomes, e.g. more efficient CO_2 fixation in deeper layers of spinach leaves under green light (Bulley et al., 1969; Clark & Lister, 1975; Sun et al., 1998). Overall, the contradictions of the many different studies concerning the spectral efficiency of green light are still a disputed topic and it seems as if the efficiency, with which different plants can use different light spectra, is species dependent.

A review by Wang et al. (2014) concerning the influence of light quality on growth in microalgae showed that overall, red light is favourable for effective growth, which is supposedly caused by improved absorption by light harvesting pigments. Ooms et al. (2017) tested the influence of light quality on the photosynthetic cyanobacteria

Synechococcus elongatus in photobioreactors, where they were exposed to different light spectra at light with intensities of 50 µmol m^{-2} s^{-1} and 2000 µmol m^{-2} s^{-1}. In low-density cultures with low light intensities, red light clearly outperformed other light spectra. With increasing biomass densities and light intensities, the productivity of green light was four times higher than that of red light. This aligns with previous observations, which implied that red light is best used for microalgae cultivation at low densities (Baer et al., 2016; Wang et al., 2007). When using green light at low intensities, most of it is lost due to poor absorbance (Ooms et al., 2017). At high intensities however, green light offers two very distinct advantages. Because of its low absorbance, it can penetrate much deeper into the algae solution and hence performs better than other light spectra, where inter-cell shading leads to light attenuation (Ooms et al., 2017). Additionally, because of the weak light absorbance per cell, the detrimental effects of photoinhibition are lessened (Ooms et al., 2017). In other words: The absorbance of red light per cell is high, which increases the shadowing effect in dense cultures and can quickly lead to light stress in the absorbing cells. The absorbance of green light on the other hand is low per single cell, but therefore high for the whole culture, because the light is deeply and evenly entering the culture without causing photoinhibition.

3.2.3 Chlorophyll fluorescence

Photosynthesis in plants is located in the chloroplasts, where two photosystems, photosystem I and photosystem II (PSII), anchored in the thylakoid membrane, collect light and are using the energy that it provides for chemical work, generating NADPH and ATP for later biomass fixation (Heldt & Piechulla, 2010). These photosystems contain pigments like chlorophyll, carotenoids and xanthophylls, which collect light in antenna complexes. While chlorophyll b mainly enhances light absorption,

chlorophyll a is a constituent of the reaction centres in the photosystems and is therefore regarded as the main pigment of photosynthesis (Heldt & Piechulla, 2010). When photons reach a chlorophyll molecule, an electron in the conjugated system of double bonds in the molecule is excited to a higher orbit, termed a singlet. Electrons from the first singlet, also called first excitation state, can be used for chemical work in the process of photosynthesis, but the level of excitation is dependent on the light spectra of light reaching the pigment. Light of red wavelength has a lower amount of energy and will excite electrons from the ground state to the first excitation state. Blue light on the other hand has more energy, exciting electrons to the second singlet, which is too unstable to be used for chemical work. The excess energy is then emitted as heat and fluorescence to reach the first singlet (Heldt & Piechulla, 2010). Chlorophyll fluorescence therefore happens, when the energy of the photon reaching the pigment is too high, but it can also happen when too many photons are reaching the photosystem (Schreiber, 2005). After excitation of an electron by photons, the electron passes down an electron transport chain, where water is split into oxygen and hydrogen. This ultimately reduces NADP to NADPH and creates a proton gradient for the synthesis of ATP (Heldt & Piechulla, 2010). However, this electron transport chain requires electron donors and acceptors related to the PSII. If the PSII is oversaturated with light induced energy, it reaches a state of being ‘closed’, meaning the downstream electron acceptors have not yet passed on their electron and are therefore unable to accept more. The excess energy is then again dissipated as heat or fluorescence to prevent it from causing harm (Schreiber, 2005).

Fluorescent light has the characteristic of being of a longer wavelength than the light inducing it. It can be used as an indicator for the viability of photosynthetic organisms and is typically measured by the usage of a pulse amplitude modulation chlorophyll

fluorometer (in the following referred to as ‘PAM’) (Schreiber, 2005). This method uses the principle of applying a saturating light pulse of a known wavelength with a constant pulse amplitude onto the sample and then using a photodiode detector to register chlorophyll fluorescence (Schreiber, 2005). The detector of the measuring system is tuned to only detect fluorescence caused by the measuring light, which means this method can even be used with background illumination or under full sunlight in the field (Maxwell & Johnson, 2000). The result of this measurement is an indicator to what extent the PSII uses the absorbed energy and provides information about light stress. The information it gives is therefore valuable, but no absolute measurement of photosynthesis (Maxwell & Johnson, 2000).

3.2.4 Acutodesmus obliquus

Acutodesmus obliquus (Turpin), formerly *Scenedemus obliquus,* is an ellipsoidal, spindle shaped freshwater microalgae with acute poles (Krienitz & Bock, 2012). It can be observed in flat or curved coenobia of two to eight individuals or solitary (Choi et al., 2015; Krienitz & Bock, 2012) (see figure 2). *A. obliquus* can tolerate high pH values, however its optimum pH is around neutral conditions (Goldman et al., 1982), and its optimum growth temperature is around 30 °C (Hindersin, 2013). Piotrowska-Niczyporuk et al. (2015) proposed *A. obliquus* as a model organism for heavy metal studies and has shown that under heavy metal toxicity of lead, cells of *A. obliquus* are able to detoxify heavy metals by the usage of both enzymatic and non-enzymatic antioxidants. The suitability of *A. obliquus* for the production of biofuels, especially biodiesel from triacylglycerols (TAGs), has been tested and was reckoned as high, which was mainly due to its overall high productivity (Abomohra et al., 2013; Breuer et al., 2012). Its biomass production was 2.5 times higher than in other species during growth under nitrogen starvation, which is a stressor used to enhance TAG production

in microalgae (Breuer et al., 2012). Remmers et al. (2017) found that starving the microalgae after they were grown under nitrogen supply for a period of time gives a is two times higher time-averaged yield of TAG than growing the algae under continuous nitrogen limitation. However, they also found that starch is the primary storage metabolite and only a limited synthesis rate will lead to higher production of TAGs as storage metabolites. Abomohra et al. (2013) tested different algae species to find a suitable candidate which has a high rate of lipid and fatty acid production, while also having high growth rates in inexpensive nutrient media. The findings of the study were that the lipid and FA content of *A. obliquus* were low compared to other species with 19 % and 10 % respectively. They still deemed *A. obliquus* to be a suitable candidate for large scale production, because it had by far the highest growth rates, which mitigated the low lipid content. An overall high lipid productivity was reached while it was possible to grow *A. obliquus* in a cheap medium (Abomohra et al., 2013; Hindersin et al., 2013).

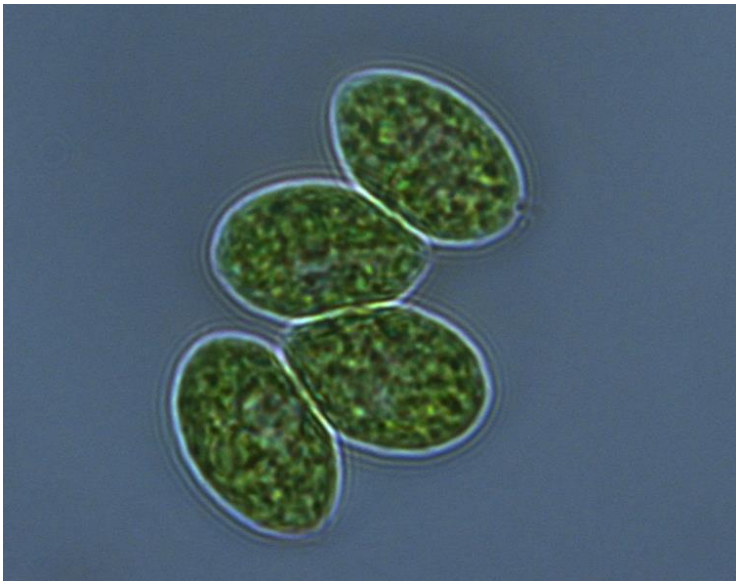

Figure 2: Picture of coenobia of four Acutodesmus obliquus *cells, taken by Astrid Lindner at the Leuphana University Lüneburg.*

3.2.5 Fatty acid profiles developed under different light spectra in microalgae

To maximise lipid production from microalgae for industrial uses, the factors influencing their production need to be known, especially if the aim is to specifically produce targeted fatty acids. It has been shown that light, temperature, as well as the concentration of CO_2 and nutrients can influence the lipid composition in different microalgae (Guschina & Harwood, 2006). Changes in fatty acid profiles are means of organisms to influence their protoplastic viscosity to guarantee normal metabolic processes at different temperatures (Lewis, 1962). But while temperature is in general influencing the FA profile, it seems as if the expected increase in unsaturation with lower temperatures is not always happening (Guschina & Harwood, 2006). This shows that in some cases, the correlation of more unsaturated fatty acids with decreasing temperature is too simple and other factors have a greater impact. Concerning quantity of fatty acids, it seems as if stressors like nitrogen depletion are decisive for the accumulation of TAGs (Hu et al., 2008). However, an increased accumulation of lipids is also correlated with decreased growth, which is why stressors should not be used as means of increasing lipid accumulation (Francisco et al., 2010). As photosynthetic organisms, microalgae are heavily influenced by the quality of light, meaning its intensity and the included light spectra they are receiving (Choi et al., 2015; Shu et al., 2012). One idea was to use this variable to try and influence growth and lipid profiles. Shu et al. (2012) tested the influence of light of green, blue and red spectral wavelengths with intensities of 500, 1000 and 2000 lux on a mixed culture of *Chlorella* spp. and *Saccharomyces cerevisiae*. The growth of algae was best under exposure to red light at an intensity of 1000 lux. In terms of oil production, blue light at the same intensity was favourable. Green light gave the lowest growth and the lowest lipid production, but also showed the lowest photoinhibition compared to red and blue light. Hultberg et al. (2014a) tested the growth and analysed the fatty acid

profile of *Chlorella vulgaris* under six different light spectra at 100 μmol m^{-2} s^{-1}. It was observed that the growth was significantly higher under red and white light conditions compared to blue and green light. In their study, green light also gave the overall lowest concentration of lipids, but the proportion of polyunsaturated fatty acids was increased in this group. The main fatty acids in *Chlorella vulgaris* were 18:3, 16:3 and 16:0, while 16:1, 16:2, 18:0, 18:1 and 18:2 were present in lower amounts. As *A. obliquus* has high growth rates in cheap growth medium and therefore a high potential for commercial production, it is of special interest to find ways to manipulate its production of lipids while not causing its growth to decrease due to stress. This study therefore had the aim to gain knowledge about growth and FA composition of *A. obliquus* grown under different light quality and temperature conditions.

4 Material and methods

4.1 Cultivation

4.1.1 Pretreatment of Acutodesmus obliquus

The freshwater microalgae *Acutodesmus obliquus* (SVCK 10169; Microalgae and Zygnematophyceae Collection Hamburg) was used for all experiments. They were kept in a monoculture in petri dishes and used to inoculate a small volume of growth medium in an Erlenmeyer flask. The growth medium contained 2 g L^{-1} Flory Basic Fertilizer 1 (Euroflor, Germany) and 3.22 g L^{-1} KNO_3 (Carl Roth, Germany) as nitrogen source, dissolved in distilled water. The same growth medium was used for all starter cultures, precultures and test cultures. The nutrient content was chosen to be high enough to assure that the algae never reached the phase of nutrient depletion during the short experiments of this study. These small starter cultures were used to inoculate a preculture, which was cultivated under constant light at a photon flux density of 150 µmol m^{-2} s^{-1} (Sylvania T9 Circline 32W) at around 25 °C. A magnetic stirrer was used to prevent sedimentation of the algae and they were aerated with CO_2 enriched air (4 %v/v). The setup can be seen in figure 3. A new preculture was established for each experiment and as they were all cultivated under the same conditions, uniformity for each experimental start was given.

Figure 3: Preculture of Acutodesmus obliquus illuminated by a white light Sylvania light bulb and kept in motion by a magnetic stirrer and pressurised air mixed with CO_2.

4.1.2 Cultivation of Acutodesmus obliquus

A purpose-built cultivation unit holding up to twelve large test tubes was used for all experiments. In this study, *A. obliquus* was cultivated in eleven test tubes with a total volume of around 4 L. For inoculation, the optical density (OD) of the preculture was measured and the required volume to establish four litres with an OD of 0.2 were calculated. An example of this calculation can be found in the appendix (10.1 Example calculation for establishment of starter cultures).

The test tubes were held up in the cultivation unit by four inlays of black acrylic glass with three test tube chambers each. The inlays were held by a transparent acrylic glass tank filled with water. The water was heated or cooled by a heat exchanger connected to the test tank. This setup assured that the temperature condition in all test tubes was similar. The open front side of the inlays exactly adjoined the acrylic glass wall of the tank and the hole in the top part was just wide enough for the test tube to fit in, so there were no gaps for stray light to reach the algae. Via a mixing bottle, humidified and CO_2 enriched air was led into the tubes by straws reaching down to the bottom of the tubes. The humidified air kept the algae in a

homogenous solution and lowered evaporation from the test tubes. The mixing bottle was filled with distilled water and pressurised air, as well as CO_2, were entering it. A schematic drawing of the experimental setup can be seen in figure 4.

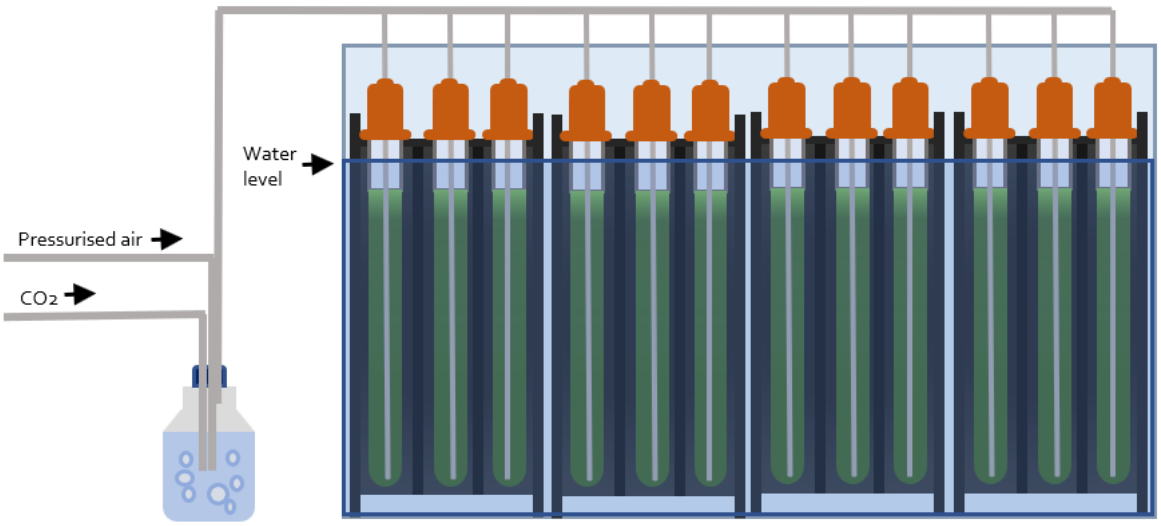

Figure 4: Schematic drawing of the experimental setup with a mixing bottle that leads air enriched in CO_2 and with a high humidity into up to twelve test tubes, in which microalgae can be cultivated.

4.1.3 Light source

Light for the growth of the algae is supplied by Philips MSR 575 HRCT metal halide lamps with a full sunlike light spectrum (see figure 7a). Their colour temperature of 6000 K is very close to the sun´s colour temperature of approximately 5900 K above the atmosphere (Matson et al., 1984; Philips, 2019). Figure 5 shows the sunlike spectrum of the lamps, notably there is no UV light reaching behind acrylic class. In this experiment, the algae received light 24 hours per day with no dark period was used.

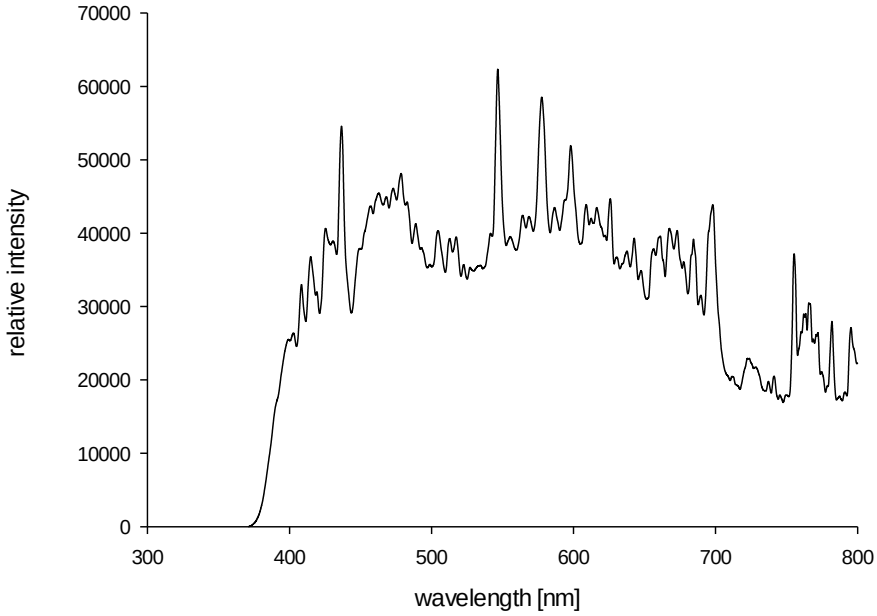

Figure 5: Light spectrum of the Phillips MSR 575 HR CT light bulb behind acrylic glass.

4.1.4 Optical filter foils

To control for the spectral light spectra reaching the tubes, optical filter foils (LEE filterfoils) were used. These foils have known absorption spectra and could be put directly onto the glass tank. A red, green and blue foil were used on three tubes each, while the rest of the tubes were used as full solar spectrum control. Figure 6 shows the experimental setup with the coloured foils put on the tank, figure 7 shows an actual photographs of the setup and the lamps used.

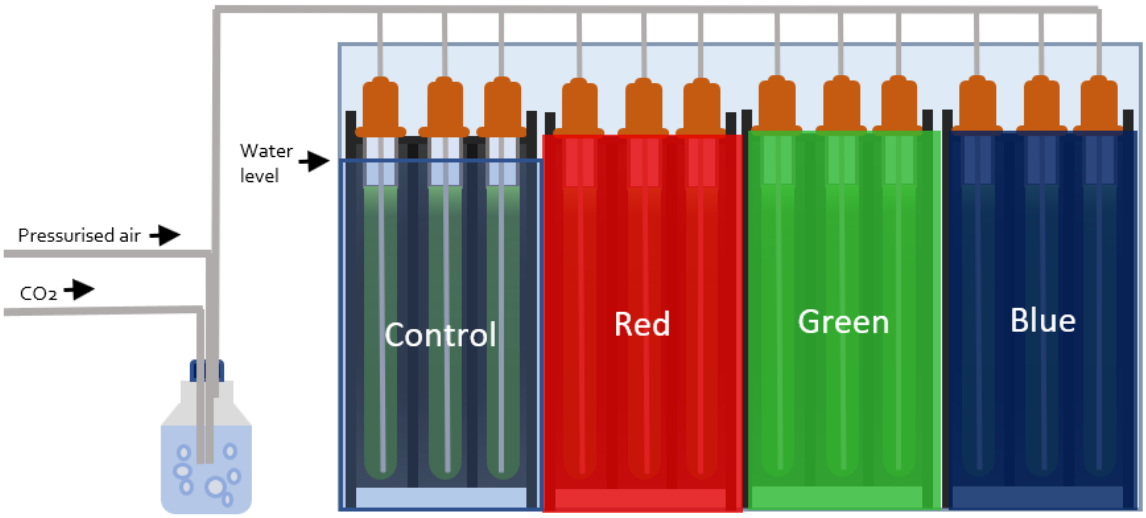

Figure 6: Experimental setup with coloured foils controlling for the light spectra reaching the test tubes.

Figure 7: Lamps used for the experiment (a) and photograph of the experimental setup (b).

4.2 Study design

Three different factors were controlled for in the experiments. The first, which was the same for all five experiments, was control of light spectra. The microalgae were exposed to photosynthetic active radiation (PAR) of three different spectral ranges, representing the red, the green and the blue light of the electromagnetic spectrum. Those were compared to a full

solar like spectrum by the usage of a white light control group. Low, moderate and high light intensities were tested, with low being at 120 µmol m^{-2} s^{-1}, moderate at 480 µmol m^{-2} s^{-1} and high intensities at 800 µmol m^{-2} s^{-1}. Each light intensity was tested at 30 °C, for the moderate intensity, additionally a colder (20 °C) and warmer temperature (35 °C) were tested. An overview of the study design is given in figure 8.

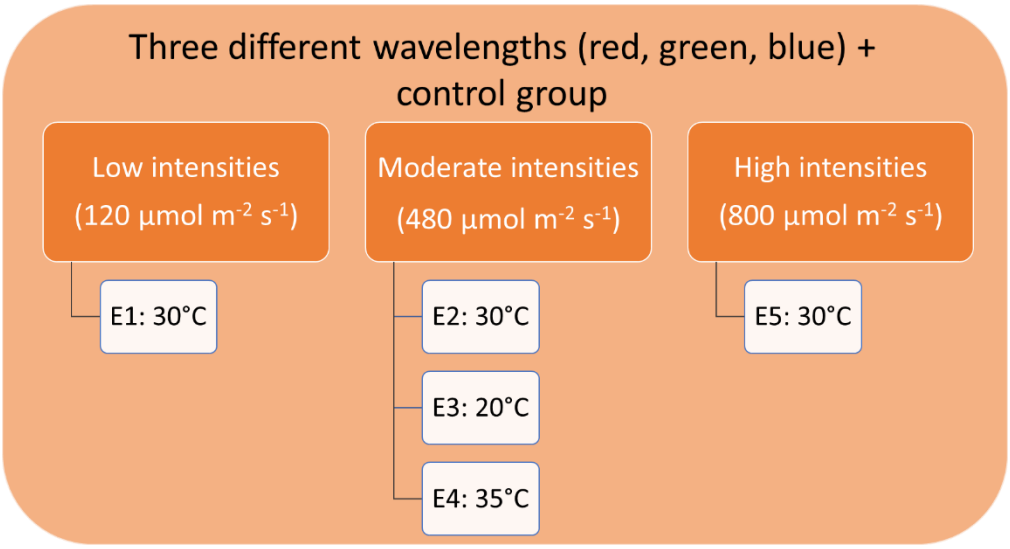

Figure 8: Overview of the study design with the general control for light spectra and the subordinated control for light intensities and temperature.

The foils used were purchased from *LEE Filters*, the three colours were 182 *light red*, 124 *dark green* and 119 *dark blue*. Figure 9 shows the spectra of the foils stated in the data sheets provided by *LEE Filter*, as well as the spectra measured behind the acrylic glass of the cultivation unit with a spectrometer (*119 Dark Blue; 124 Dark Green; 182 Dark Red*). The blue filter foil had a spectra light area from 380 nm to 540 nm, with a peak at 450 nm (see figure 9, a), the green filter foil had a spectral area from 450 nm to 650 nm, with a peak at 520 nm (see figure 9, b) and the red filter foil had a spectral area of the visible light from 580 nm to 700 nm, with a peak at 690 nm (see figure 9, c).

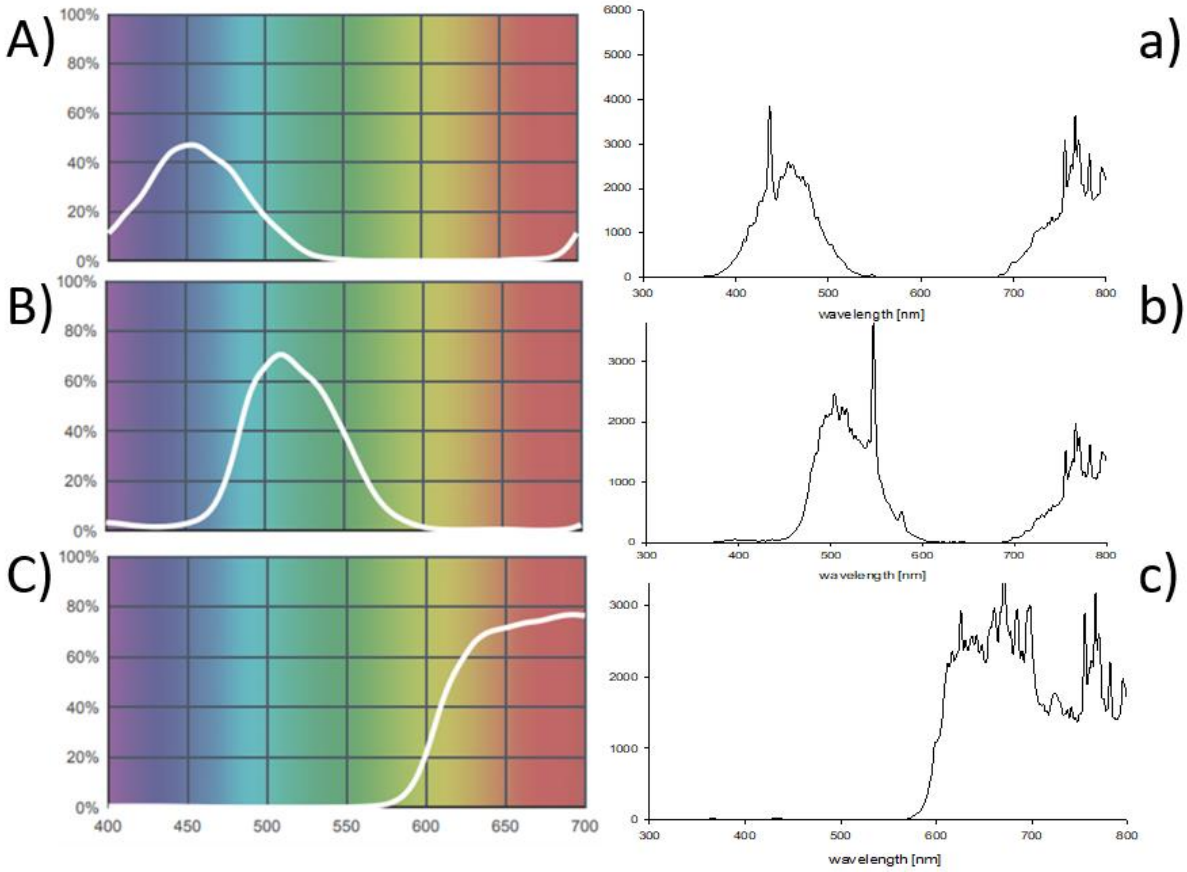

Figure 9: Absorption spectra of the three LEE filter foils provided by the distributor (119 dark blue *A), 124* dark green *B) and 182* light red *C) and measured in the lab behind acrylic glass (119* dark blue *a), 124* dark green *b) and 182* light red *c).*

4.3 Light intensities

Intensities were measured at the top, centre and bottom of the test tubes and the average for each tube was taken to determine the average for each colour group. The position of the lamps was adjusted in a way that the average of the triplicates was comparable between all colour groups. This makes the average growth comparable for the different light spectra, because they received approximately the same number of photons. Table 1 shows the values of light intensities used in these experiments, with a colour marking to show the differences. The two tubes exposed to white light are called W1 and W2, the red light triplicates are called R1, R2, R3, for green light it is G1, G2, G3 and for the blue light triplicates it is B1, B2 and B3. With the present experimental setup, it was impossible to create conditions where

light intensities are exactly the same in all tubes. However, it was possible to adjust the light in a way that the test tubes were on average alike in terms of photosynthetic active radiation. For the experiments E2, E3 and E4 however the average of the white light control group was too high, which is why here only W1 was used as reference. For E5, the intensities of the tubes W1 and B1 were too high, so they were not included in the study to keep the average values the same for all tubes.

Table 1: Light intensities in µmol m^{-2} $s^{-1}1$ for the three different sets of intensities (E1 low, E2; E3 and E4 moderate and E5 high) for each tube (W1 to B3) on average from top to bottom and the total average between the samples of each light spectrum group. Fields are colour coded, with green meaning the tube has a low intensity compared to the avergage, yellow meaning average intensity and red showing a high intensity in this tube compared to the average for this experiment.

		W1	W2	R1	R2	R3	G1	G2	G3	B1	B2	B3
E1	Average for tube [µmol m-2 s-1]	106	151	95	115	141	102	117	127	123	122	112
	Average between samples [µmol m-2 s-1]	129		117			116			119		
E2, E3, E4	Average for tube [µmol m-2 s-1]	497		397	502	508	453	490	518	472	488	487
	Average between samples [µmol m-2 s-1]	497		469			487			482		
E5	Average for tube [µmol m-2 s-1]		800	897	753	732	702	758	878		837	775
	Average between samples [µmol m-2 s-1]	800		794			779			806		

4.4 Growth and photosynthetic activity measurements

Methods used in this study during cultivation of *A. obliquus* were measurements with a pulse amplitude modulation chlorophyll fluorometer and measurement of optical density with a spectrometer.

4.4.1 Photosynthetic activity

The viability and photosynthetic activity of the algae cells were measured by a pulse amplitude modulation chlorophyll fluorometer (Dual-PAM-100, Walz, in the following referred to as PAM). In this study the effective PSII quantum yield Y(II) was measured, which is calculated from the ratio of two fluorescence values and therefore independent of

signal amplitudes (Schreiber et al., 2007). No dark adaption of the photosynthetic cells is needed for Y(II) measurements, hence it was conducted directly on the dried glass walls of the tubes. Schreiber (2005) stated that terrestrial plants usually have a Y(II) value of 0.8, while that of algae is lower. In a study with *Chlorella vulgaris*, *Phaeodactylum tricornutum* and *Desmodesmus subspicatus*, viable control groups had an average values of 0.589, 0.523 and 0.593 respectively (Schreiber et al., 2007).

4.4.2 Optical density and correlation to dry weight

Samples were taken daily at the same time, OD was then measured in a spectrophotometer (Pharmacia LKB Ultropsec III; Gemini) and correlated to dry weight (DW). In a spectrophotometer, the absorbance of a solution is measured, which means the decrease of light transmitted through a solution containing particles, compared to a blank only containing the suspending liquid (Clesceri et al., 1998). The pigments present in plant cells play an important role in the absorbance of those cells. Griffiths et al. (2011) recommend taking certain precautions to assure a low error level for dry weight estimates from optical density measurements of plant cells. This includes using a wavelength for measurements that lies outside the ranges of absorbance for the main pigment chlorophyll, so in this case a wavelength of 750 nm was used during the daily OD measurements. The correlation to DW was established by measuring the OD of linearly diluted mixtures from at least one tube from each colour. Triplicates were measured for optical density, then a known volume of each dilution was applied onto nylon filters (Celluloseacetate filter membranes with a pore size of 0.45 µm) with a known dry weight, using a vacuum pump for removal of water. Subsequently, the filters were dried overnight at 80 °C and their weight was measured and divided by the used volume. Dividing the average OD of the dilutions through their average dry weight gives the correlation value for optical density to dry weight (OD-DW). For most

of the experiments, the value was just above 2, an example calculation can be seen in the appendix (10.2 Example calculation for optical density to dry weight correlation).

4.5 Lipid extraction and derivation

Every day, a specific sample was drawn from the test tubes and then stored in a freezer (-20 °C) prior to lipid extraction. A modified Folch lipid extraction as described by Reich et al. (2012) was used for preparing the samples for GC-MS (Folch et al., 1957). The required sample volume was calculated for a targeted dry weight of 2.5 mg (See appendix, 10.3 Example calculation for required volume for lipid extraction). All extraction steps were performed in glass tubes to avoid contamination with FAs used in the production process of plastic tubes. The samples in the glass tubes were centrifuged at 3200 rpm for around five seconds. The supernatant water was discarded and 20 µl of an internal standard of heptadecanoic acid solved in n-hexane (1 mg/ml) were added to the pellet. Afterwards, 4 ml of chloroform methanol (2:1) were added and the tubes were vortexed until the pellet was fully dissolved. The glass tubes were centrifuged again and the upper phase, containing the extracted lipids, was transferred to a second glass tube (Figure 10, a). The pellet in the first glass tube was treated with methanol chloroform again and vortexed again. It was then put on an orbital shaker (KS 501 Digital, Thermo Fisher) (Figure 10, b). This procedure was repeated three times and the samples were incubated for one, three and twelve hours on the orbital shaker. The previously green pellet should be of an eggshell like colour afterwards and was discarded (Figure 10, c).

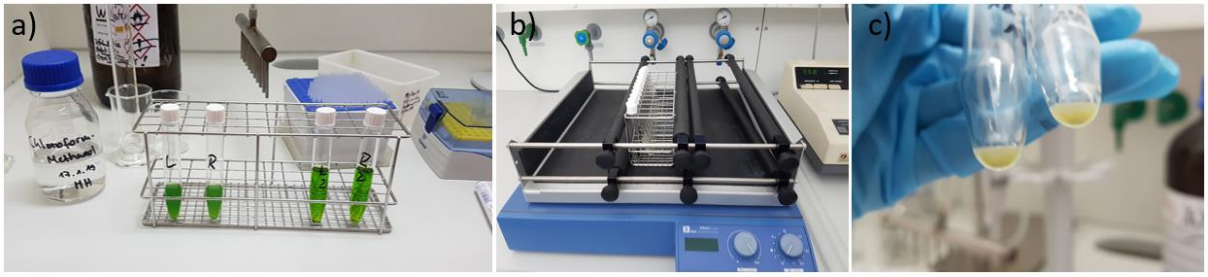

Figure 10: Lipid extraction in chloroform methanol (2:1) (a), samples on the orbital shaker (b) and a three times extracted, discoloured pellet (c).

To begin derivatisation to fatty acid methyl esters (FAMEs), the chloroform methanol extract containing all FA based lipid fractions was put under a nitrogen flow to evaporate the solvent. The dry residue was resuspended in 200 µl chloroform, 2 ml methanol and 100 µl hydrochloric acid (32 %). The solution was transferred to a headspace vial and covered under a nitrogen atmosphere before being transesterified at 100 °C for one hour in a thermal shaker (Q-101, BÜCHI Syncore) (Figure 11, a). This acidic catalysis produces FAMEs, which helps with the chromatographic separation in the GC-MS. The last step was the addition of 2 ml Millipore water and 2 ml hexane. After vortexing the solution, a clear upper and lower phase emerged with the upper phase containing the FAMEs ready for analysis via GC-MS (Figure 11, b).

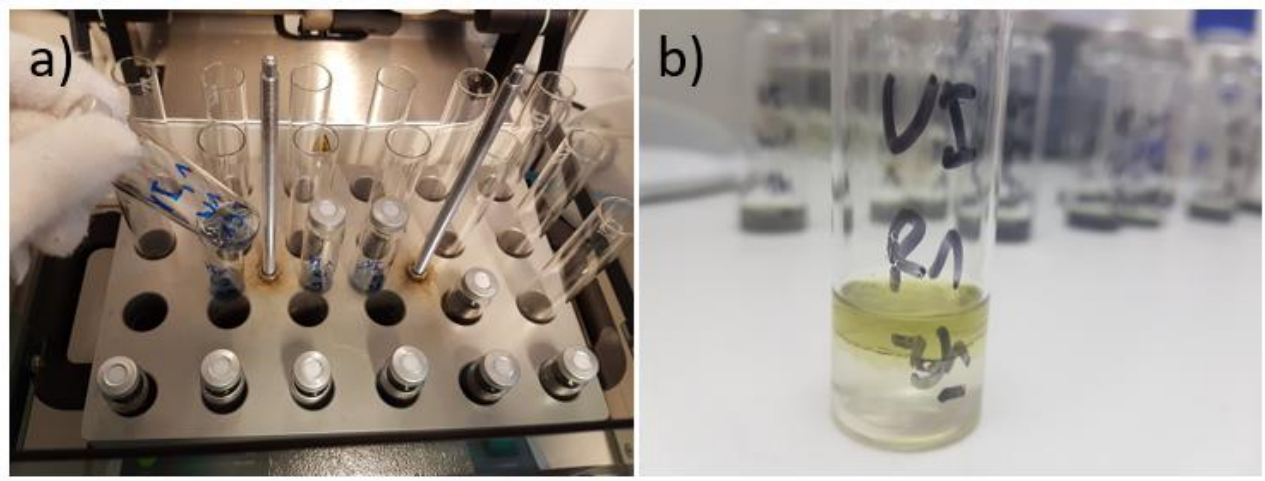

Figure 11: Samples in headspace vials in a thermal shaker (a) and separated phases with lipids dissolved the upper hexane phase (b).

4.6 Analysis

4.6.1 GC-MS for analysis of fatty acid profiles

A FAME GC-MS column (ThermoFisher) was used for the analysis of fatty acid methyl esters (FAMEs) in this work. It has a length of 30 m, a width of 0.25 mm and a coating thickness of 0.25 µm. A volume of 1 µl was injected and heated up to 260 °C in the inlet. The column is equipped to tolerate temperatures up to 260 °C, which it reaches after around 36 minutes. The temperature program takes 40 minutes and starts with 60 °C, which can be seen in figure 12. The temperature of the transfer line to the MS part is 260 °C and the temperature of the ion source is 270 °C. Electron ionisation is used in this MS and the mass range is 40 amu to 600 amu. For analysis, peak area ratios of fatty acids compared to the peak area of the internal standard were established and compared.

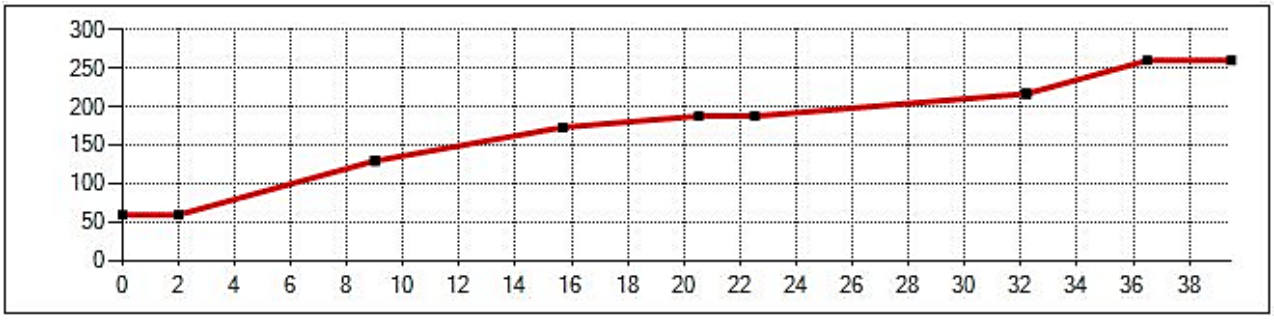

Figure 12: GC-MS temperature program for analysis of fatty acids methyl esters.

4.6.2 Data analysis

Microsoft Excel was used for storage and editing of data as well as for creating graphs. The program Graph Pad InStat was used to conduct t-tests for growth and fatty acid profile results, to test if differences were significant. This was only conducted for sample categories which included triplicates. Differences were considered as significant at $p < 0.05$. For the fatty acid composition analysis, only the time point of 96 hours was tested in terms of analysis and only fatty acids that were usually present in amounts of more than 5 % (16:0,

16:3, 16:4, 18:1, 18:2, 18:3) were analysed. Analysis of variance (ANOVA) was conducted with the data analysis add-in of Microsoft Excel, differences were considered significant at $p < 0.05$.

5 Results[1]

5.1 Growth results

The white light control group performed best in every experiment. The red light group was similar to, or higher than, the green light, but as p-values are showing, often not significantly (for p-values refer to appendix, 10.5.2 Significance tables growth). For the experiment E1 at low intensities, growth in the blue and green light group was similar with no significant differences, except at 24 hours ($p = 0.03$). The increase of biomass in the green light group was significantly higher than in the blue light group in the experiments with moderate intensities. In E5 at high intensities and 30 °C, growth of the green and red light was similar until 72 hours after the start of the experiments, but after 96 hours, the green light group tended to have higher growth, however not significantly higher ($p = 0.07$)

Table 2 shows the average dry weight in gram per litre for each colour group after 96 hours of cultivation, together with standard deviations.

Algae growth during experiment E1 (low intensities, 30 °C) was low compared to experiments at higher intensities. The biomass values for all colour groups in E1 had significantly lower values than their counterparts in experiments using higher intensities (for p-values from t-tests see appendix, 10.5.2 Significance tables growth). At 96 hours, the highest dry weight in the white light control group was 0.92 g L^{-1} and the lowest dry weight in the blue light group was 0.36 g L^{-1}. Green light was only marginally higher with 0.41 g L^{-1}. The green light group had a significantly higher dry weight value in experiment E5 (high intensities), than in experiment E2 (moderate intensities) ($p = 0.009$).

[1] In the following, many coloured graphs are presented with the colours red and green often being present next to each other. Please note that red lines and bar graphs are always textured for easier differentiation for people with colour vision deficiency.

For the set of experiments with moderate intensities (E2, E3 and E4), experiment E2 with a temperature of 30 °C gave the highest biomass values for the algae (white light group 3.35 g L^{-1} and blue light group 1.59 g L^{-1}) compared to temperatures of 20 °C (E3) and 35 °C (E4). The high temperatures in E4 led to a growth lower than at 30 °C, but still higher than at 20 °C in E3 (for E4 white light group 2.74 g L^{-1} and blue light group 1.34 g L^{-1}, for E3 white light group 2.38 g L^{-1} and blue light group 1.22 g L^{-1}). While the biomass values for the colour groups are significantly different between cultivation temperatures of 20 °C and 30 °C, the differences are only significant in the green light group comparing cultivation temperatures of 30 °C and 35 °C (p = 0.05, rest of p-values see appendix, 10.5.2 Significance tables growth).

In E5 (high intensities at 30 °C) the biomass values were highest for all colour groups, ranging from 4.56 g L^{-1} in the white light group to 2.42 g L^{-1} in the blue light group.

Table 2: Average dry weight values in g L^{-1} with standard deviations in g L^{-1} for experiments E1 to E5 after 96 hours.

	Temperature in °C	Intensity in µmol m^{-2} s^{-1}	white	red	green	blue
E1	30	120	0.92 (0.06)	0.58 (0.06)	0.41 (0.02)	0.36 (0.03)
E2	30	480	3.35 (0)	2.42 (0.23)	2.14 (0.09)	1.59 (0.01)
E3	20	480	2.38 (0)	1.91 (0.17)	1.59 (0.11)	1.22 (0.02)
E4	35	480	2.74 (0)	2.12 (0.12)	1.86 (0.05)	1.34 (0.04)
E5	30	800	4.56 (0)	3.39 (0.26)	3.58 (0.34)	2.42 (0)

5.1.1 Detailed comparison of growth patterns in experiment E1 to E5

Biomass production of experiment E1 (Low intensities at 30 °C) is depicted with a standardised dry weight scale to enable comparison among treatments (E1 to E5) (Figure 13). White light gave the best growth results, followed by red light, but the overall growth was low. The growth in the red colour group was significantly higher than in the green and blue colour group, except for the last day of the experiment at 166 hours (p-values from

t-tests see appendix, 10.5.2 Significance tables growth). Algae growth in the blue and green light group was similar and not significantly different, except at 24 hours.

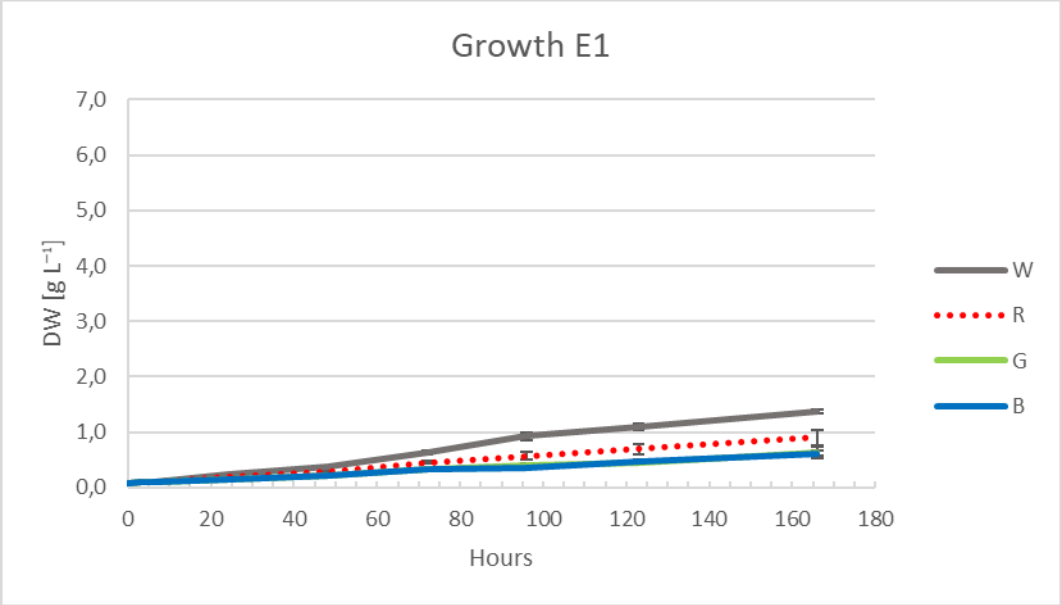

Figure 13: Comparison graph of experiment E1 (low intensities at 30 °C) with a standardised scale. Growth curves in g L^{-1} DW for a time of 180 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

For E2 (moderate intensities at 30 °C), the increase in biomass was significantly higher in the red colour group than in the green and blue colour group (p-values from t-test see appendix, 10.5.2 Significance tables growth) (Figure 14). Starting at 96 hours, the values of the red colour group were not significantly higher than those of the green colour group. In contrast to E1, the green colour group had a significantly higher growth than the blue colour group after 48 hours.

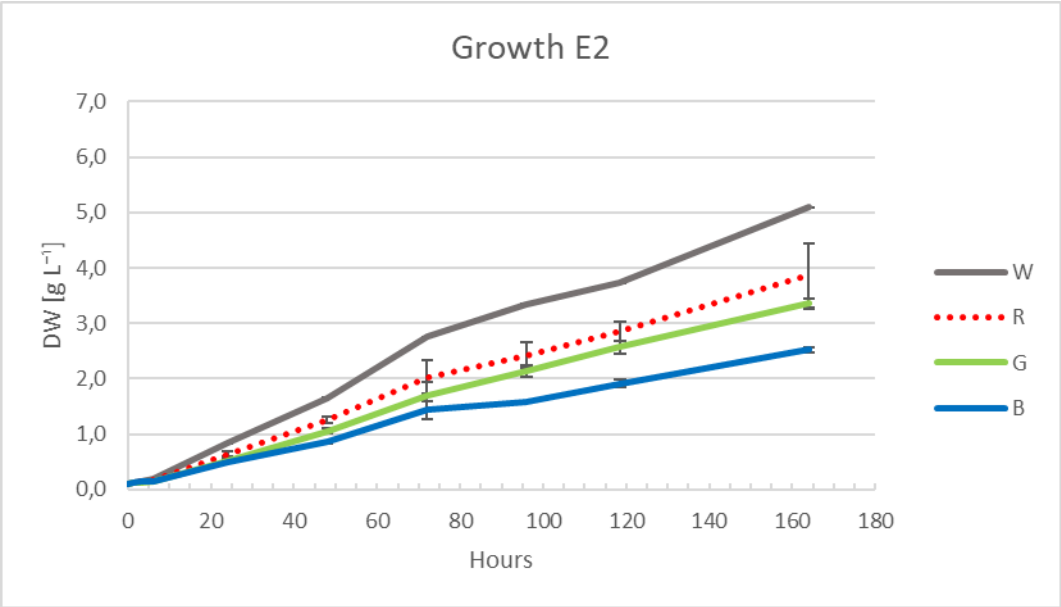

Figure 14: Comparison graph of experiment E2 (moderate intensities at 30 °C) with a standardised scale. Growth curves in g L^{-1} DW for a time of 180 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

The increase in biomass of the different colour groups in experiment E3 was lower than in experiment E2 (Figure 15). Algae growth under green light was significantly higher than it was under blue light (p-values from t-tests see appendix, 10.5.2 Significance tables growth). While the red light group had higher growth rates than the blue light group, its biomass increase was not significantly higher than in the green light group.

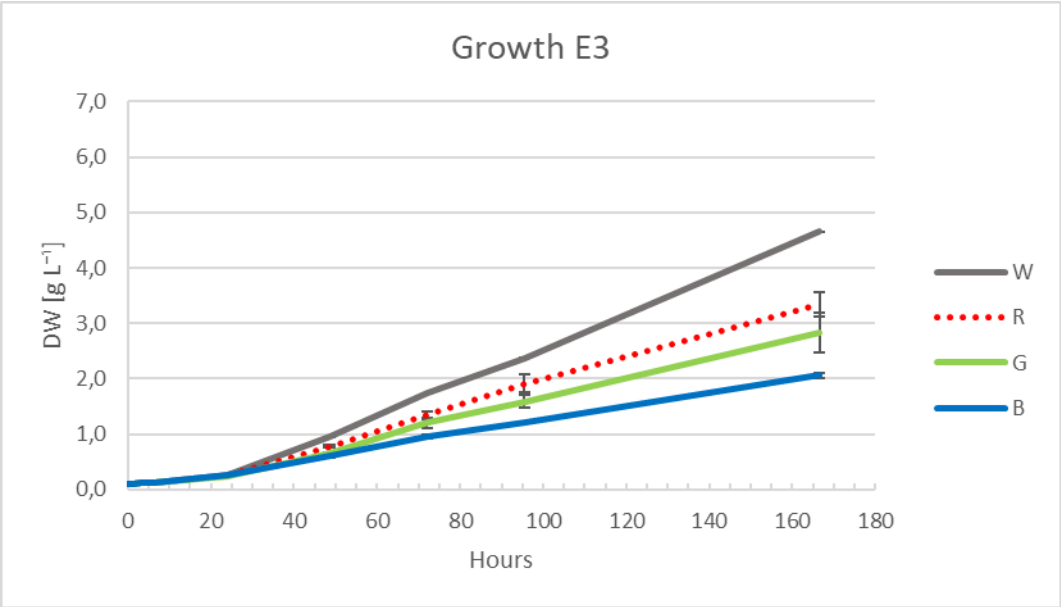

Figure 15: Comparison graph of experiment E3 (moderate intensities at 20 °C) with a standardised scale. Growth curves in g L^{-1} DW for a time of 180 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

E4 was performed at moderate intensities and high temperatures (35 °C). The growth curve is shown in figure 16. Overall the growth of all colour groups in this experiment was higher than for the experiments E1 and E3, but not as high as in E2 and E5. The differences between growth in the different colour groups were similar to the ones in E2 and E3.

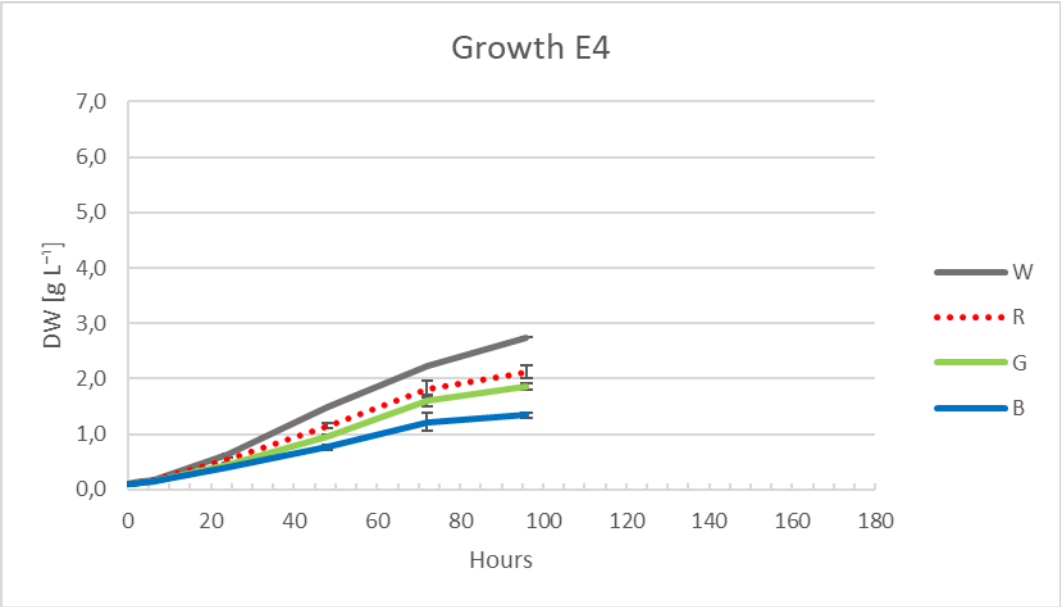

Figure 16: Comparison graph of experiment E4 (moderate intensities at 35 °C) with a standardised scale. Growth curves in g L^{-1} DW for a time of 180 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

For E5, conducted at high intensities and 30 °C, two graphs were made because the experiment was conducted for a longer time period than all other experiments (which gave rise to interesting results but required a larger scale for dry weight). The comparison graph, figure 17, shows that the overall growth of all light groups was higher than in previous experiments (E1 to E4). Notably for this experiment, the green light group had a higher biomass increase after 96 hours than the red light group. The biomass contents of the red and blue light group were however not significantly different (for p- values from t-tests refer to appendix, 10.5.2 Significance tables growth). Furthermore, it could be observed that starting at 216 hours, the green light group reached the same biomass level as the white light control group (Figure 18).

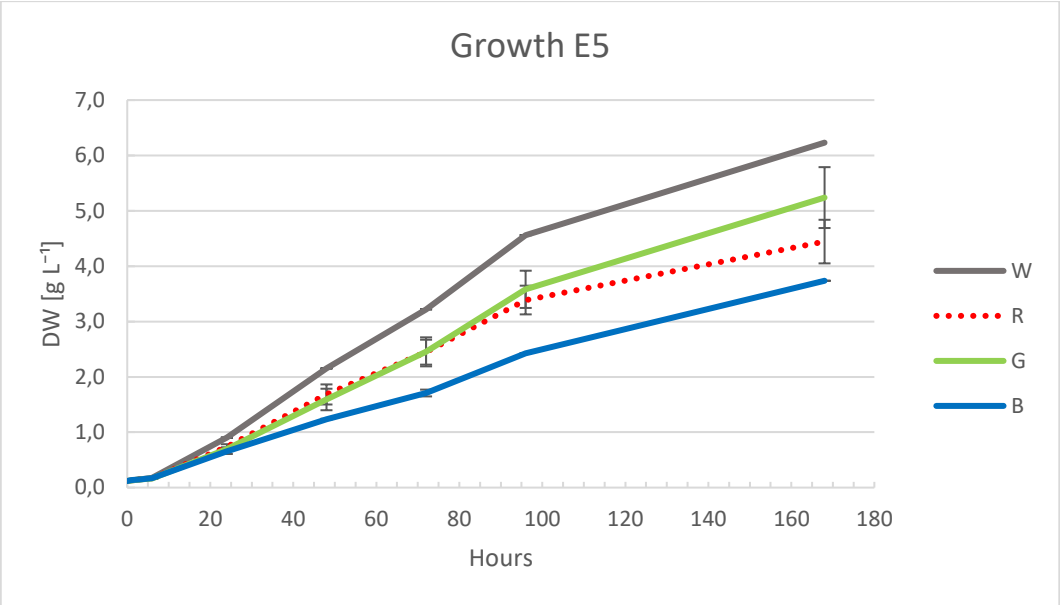

Figure 17: Comparison graph of experiment E5 (high intensities at 30 °C) with a standardised scale. Growth curves in g L^{-1} DW for a time of 180 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

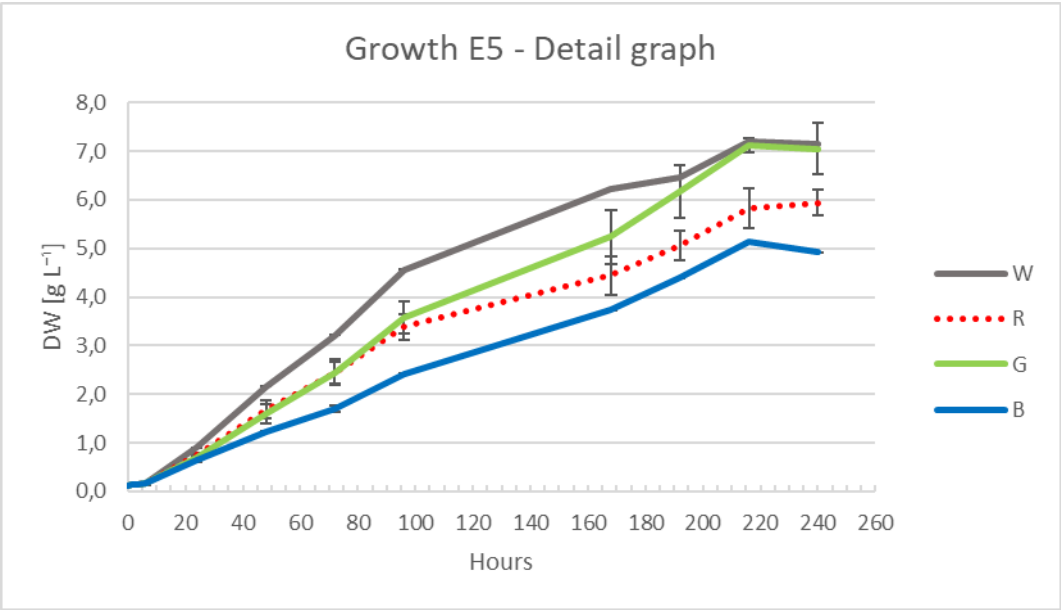

Figure 18: Detail graph of experiment E5 (high intensities at 30 °C) with an adapted DW and time scale. Growth curves in g L^{-1} DW for a time of 260 hours for the white light control group ('W', grey). red light group ('R', red). green light group ('G', green) and blue light group ('B', blue) with standard deviation bars.

5.1.2 Photosynthetic activity

The effective PSII quantum yield Y(II) for the experiments E2 to E5 was measured by the usage of a PAM. Figure 19 shows the average values for each colour group and their standard deviation at day four after the start of each experiment for E2, E3, E4 and E5. The values are ranging between 0.561 and 0.636. The colour groups did not significantly vary from each other, with the only exception of the Y(II) value of the blue colour group being significantly lower than the Y(II) value of the green colour group in E5 ($p = 0.005$; for all other p-values from t-tests refer to appendix, 10.5.2 Significance tables growth).

In experiment E2 (moderate intensities at 30 °C), the red light group had the highest Y(II) value of 0.609 (0.008). The algae solution cultivated under blue light had the lowest value of 0.561 (0.021), which was lower than the white and red light group.

The white, green and red light group in experiment E3 (moderate intensities, 20 °C) had similar Y(II) values, with the one of the green colour group being highest for this experiment with 0.602 (0.010). The blue light group with a Y(II) value of 0.589 (0.005) was performing significantly worse than green. Overall, E2 gave the most comparable Y(II) values for the four experiments.

The green light group performed best in experiment E4 (moderate intensities. 35 °C) with a Y(II) value of 0.596 (0.005). The values for the white light control group and the blue light group were lower than those of green and red. In accordance with all other experiments, values for the blue light were again the lowest for this experiment with 0.559 (0.017).

Experimental conditions of high intensities at 30 °C (E5) showed the highest Y(II) values, with green reaching 0.636 (0.003). The algae of the blue light group are performing worst, with a Y(II) value of 0.587 (0.007).

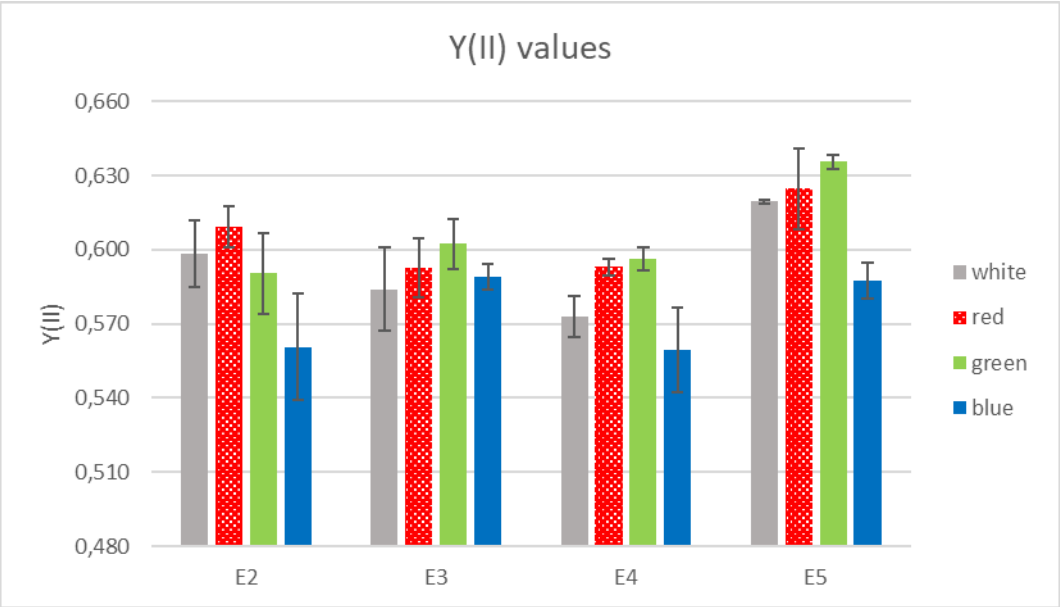

Figure 19: Values for effective PSII quantum yield Y(II) at day four after the start of each experiment for the experiments E2, E3, E4 and E5. separated into the different colour groups (red, green, blue) and the white control group with standard deviation bars.

A two-factorial ANOVA gave no significant p-values when comparing experiments with the same intensities at different temperatures, which were E2, E3 and E4 ($p = 0.4$ for temperature and $p = 0.08$ for colour). When comparing E2 and E5, experiments with the same temperature but different intensities (moderate and high), both the intensity and colour had an effect ($p = 0.025$ and $p = 0.05$, respectively).

5.2 Fatty acid profiles

All identified fatty acids of *A. obliquus* and their average percentages are shown in table 3. The average percentages were calculated using the fatty acid profiles of all precultures and all samples from each of the five experiments. FAs that were found in amounts of less than 1 % were not discussed further in this study and they are marked with a '*' in table 3. The 17:0 fatty acid present in the chromatograms of the GC-MS was not included in the table, as it was the internal standard. The 16:1 and 18:3 fatty acid were present in two isomeric forms (*cis* and *trans*), their percentages were summed for the analysis. The 16:0 FA makes up

nearly half of the fatty acids of *A. obliquus*, with an average of 43.6 % for all experiments. It is followed by 18:3, which makes up 20.9 %. The 16:4, 18:1 and 18:2 FAs are present in moderate amounts (5 - 10 %) and 16:1, 16:2, 16:3, 18: 0 and 18:4 in low amounts (0.8 - 5 %).

Table 3: Fatty acids found in Acutodesmus obliquus *with fatty acid nomenclature, trivial name and position of the first double bond. Fatty acids not included in the comparative analysis are marked with a '*', average percentages and their standard deviations were established from all precultures and all samples of each of the five experiments.*

Fatty acid nomenclature	Trivial name	Position of first double bond	Average percentage
12:0	Lauric acid		*
14:0	Myristic acid		*
15:0	Pentadecanoic		*
16:0	Palmitic acid		43.6 % (4.5 %)
16:1	Palmitoleic acid	n-7	2.1 % (0.8 %)
16:2	Hexadecadienoic acid	n-4	0.8 % (0.3 %)
16:3	Palmitolinolenic acid	n-3	4.6 % (1.4 %)
16:4	Palmitidonic acid	n-3	6.0 % (2.5 %)
18:0	Stearic acid		2.7 % (1.1 %)
18:1	Oleic/Elaidic acid	n-9	8.5 % (2.7 %)
18:2	Linoleic acid	n-6	7.7 % (2.5 %)
18:3	α-Linolenic acid	n-3	20.9 % (5.7 %)
18:4	Stearidonic acid	n-3	1.9 % (0.6 %)
22:0	Behenic acid		*
24:0	Lignoceric acid		*

For each experiment (E1 to E5), the preculture used to inoculate the test tubes and samples after six, 24 and 96 hours of growing in the test tubes were extracted and analysed.

A two-factorial ANOVA showed no significant differences between the averages of the FA profiles of the precultures ($p = 0.99$). As the precultures were similar in terms of their fatty acid profiles, it can be concluded, that differences in the FA profiles of the experiments were due to effects caused by different experimental conditions.

5.2.1 Temperature comparison

After analysis of the profiles, fatty acids were categorised into the groups ‘saturated’ (16:0 and 18:0). ‘monounsaturated’ (16:1 and 18:1) and ‘polyunsaturated’ (16:2, 16:3, 16:4, 18:2, 18:3 and 18:4).

For the following results, the experiments are arranged in the order of lowest to highest temperature. E3 (20 °C) is described first, E2 (30 °C) second and E4 (35 °C) last. At low temperatures (E3), the amount of PUFAs increased over time, while it decreased at higher temperatures (E2 and E4). The decrease of PUFAs was stronger at 30 °C than at 35 °C. While at 30 °C the amount of SFAs remained constant, it increased at 35 °C, therefore increasing temperatures led to an increase in SFAs. ANOVAS of the saturation groups showed that the development in saturation profiles was not significantly different between the blue, green, red and white colour group. However, the p-values also showed that the means for the saturation groups are significantly changing over time. Details ad significances are described in the following (Figure 20, figure 21, figure 22).

Changes in the saturation profiles of E3 (20 °C) could be observed after six hours of cultivation. There was an increase in saturated and unsaturated FAs and a decrease of PUFAs for all colours (Figure 20). After 96 hours, the percentages for SFAs were comparable to the preculture (40.4 % in preculture and on average 39.7 % (0.6 %) for the colour groups). There was a decrease in MUFAs compared to the preculture (10.3 % to an average of 7.3 % (2.5 %)), with the only exception being the red light group (11.7 %). After 96 hours it had nearly double the amount of monounsaturated fatty acids (MUFAs) of the other colours and it was

the only colour group, that had a lower amount of PUFAs than the preculture (red with 46.8 % compared to 48.3 % in the preculture). All other colours had increased amounts (around 53.5 % average for blue, green and white, which gives a total average of 51.9 % (2.9 %)). In each saturation group of E3, the p-values are showing that the changes in saturation profiles were not influenced by colour (p_{colour} between 0.1 and 0.9 for the saturation groups), but by time (p_{time} between 0.0001 and 0.004).

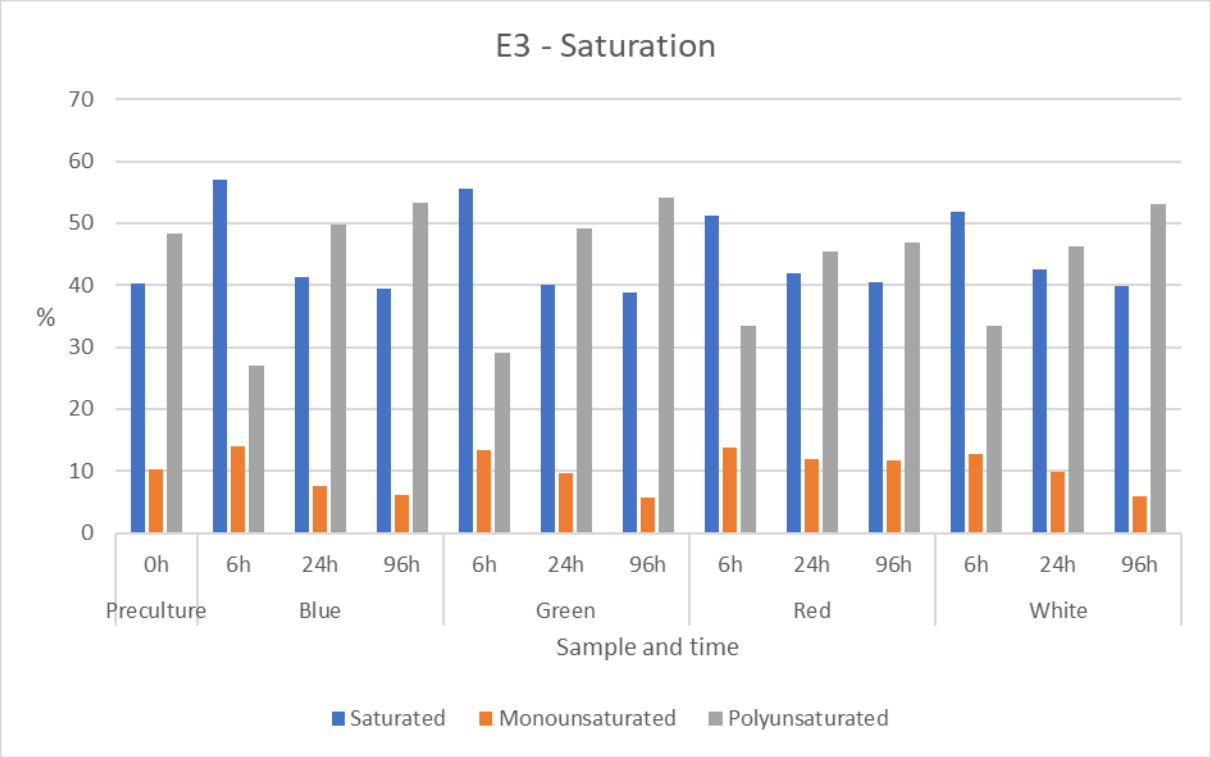

Figure 20: Saturation profile of experiment E3 (moderate intensities at 20 °C) differentiated into saturated, monounsaturated and polyunsaturated fatty acids for the preculture, the blue, green and red colour group and the white light control group at the time points of 6, 24 and 96 hours after start of cultivation.

In experiment E2 (30 °C), the amount of SFAs in the colour groups first increased compared to the preculture (42 %), then decreased, and then increased again to an average of 49.5 % (3.7 %) (Figure 21). Even though the numbers seem to vary, the p-values of the ANOVA show that neither the effect of colour nor of time is significant (p_{colour} = 0.7, p_{time} = 0.3). Except for the red light group, all other colours showed a decrease of MUFAs compared to the preculture (10.1 %), before increasing again after 96 hours to 12.8 %

(2.2 %). Both colour and time had a significant effect on the development of means of monounsaturated fatty acids (p_{colour} = 0.004. p_{time} = 0.0007). Red was increasing constantly to a value of 16.3 %. The amount of PUFAs decreased compared to the preculture (46.6 %) to an average of 36.3 % (4.7 %), without being influenced by colour or time (p_{colour} = 0.8, p_{time} = 0.1).

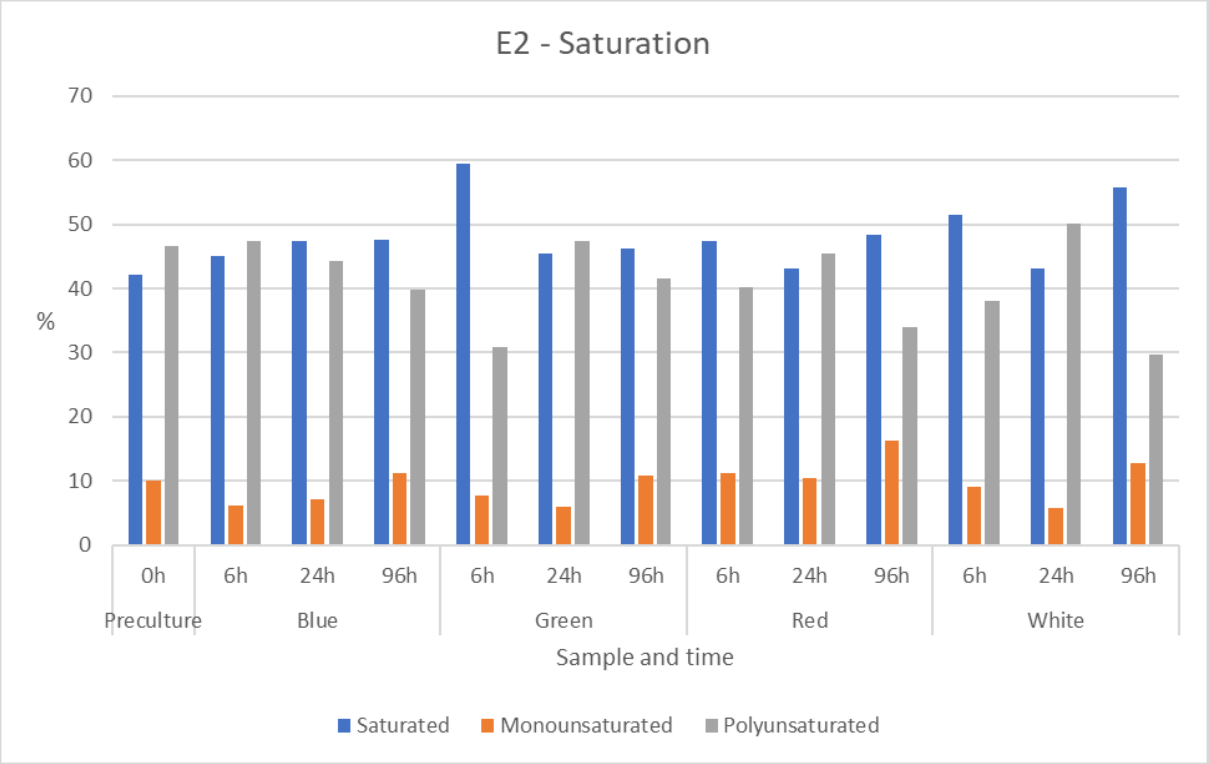

Figure 21: Saturation profile of experiment E2 (moderate intensities at 30 °C) differentiated into saturated, monounsaturated and polyunsaturated fatty acids for the preculture, the blue, green and red colour group and the white light control group at the time points of 6, 24 and 96 hours after start of cultivation.

In experiment E4 (35 °C), the amount of SFAs in the colour groups increased compared to the preculture (41.6 % to an average of 49 % (0.9 %)) (Figure 22). The percentage of unsaturated fatty acids first decreased, then increased again after six hours and was higher than the values of the preculture in the end of the experiment (10.4 % to 14.7 % (1.2 %)). The red colour group was an exception, it increased continuously over the whole experiment. The amount of PUFAs showed an overall reduction (47 % to 35 % (1.3 %)). Again, there

first was an increase for all colours except for red at six hours after starting the experiment. Concerning significant effects, E4 showed similar results as E3, with colour never and time always causing an effect on mean values in each saturation group (p_{colour} between 0.051 and 0.2. p_{time} between 0.0002 and 0.0005).

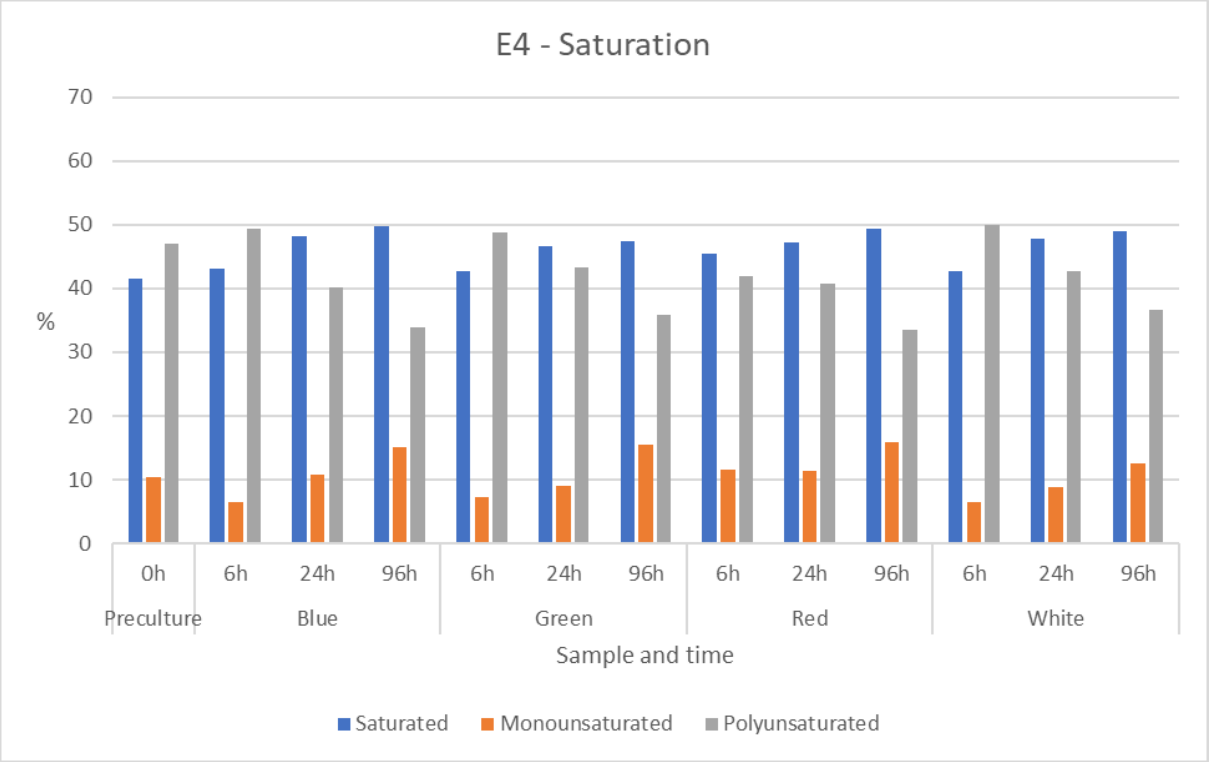

Figure 22: Saturation profile of experiment E4 (moderate intensities at 35 °C) differentiated into saturated, monounsaturated and polyunsaturated fatty acids for the preculture, the blue, green and red colour group and the white light control group at the time points of 6, 24 and 96 hours after start of cultivation.

5.2.2 Light intensity effects

The experiments performed at 30 °C under 120 µmol m^{-2} s^{-1} (E1), 480 µmol m^{-2} s^{-1} (E2) and 800 µmol m^{-2} s^{-1} (E5) all showed the same tendencies, with SFAs not changing, MUFAs increasing and PUFAs decreasing. As this trend is the same for the three different light intensities, it can be expected that those changes were due to a temperature effect, as they were all conducted at a higher temperature than the preculture. The ratios of saturated to monounsaturated and polyunsaturated fatty acids were similar for low and moderate intensities (E1 and E2). Experiment E5 gave less saturated, only half as much

monounsaturated and more polyunsaturated fatty acids in the profile of *A. obliquus*. This shows that the saturation profile was only influenced by light intensities, after they exceeded a certain threshold value higher than 480 µmol m^{-2} s^{-1}. Again, the ANOVA p-values showed that the differences in the saturation profiles were not caused by usage of the different colour groups but time. Details of E1(120 µmol m^{-2} s^{-1}) and E5 (800 µmol m^{-2} s^{-1}) are described in the following (Figure 23, figure 24).

The amount of SFAs in E1 is slightly higher than in the preculture but stays the same from six to 96 hours and is comparable for all colours (4.4 % (0.8 %)) (Figure 23). The p-values of the ANOVA conducted for this saturation group are confirming that there is no effect of colour or time (p_{colour} = 0.1, p_{time} = 0.8). While the amount of MUFAs is increasing over the 96 hours of the experiment (7.5 % in the preculture to 15.6 % (1.7 %)), the amount of PUFAs is decreasing (37.6 % (2.8 %)). Differences in mean values were caused by colour and time (p_{colour} = 0.002 for monounsaturated, 0.04 for polyunsaturated fatty acid, p_{time} = 0.0008 for monounsaturated and 0.01 for polyunsaturated fatty acids).

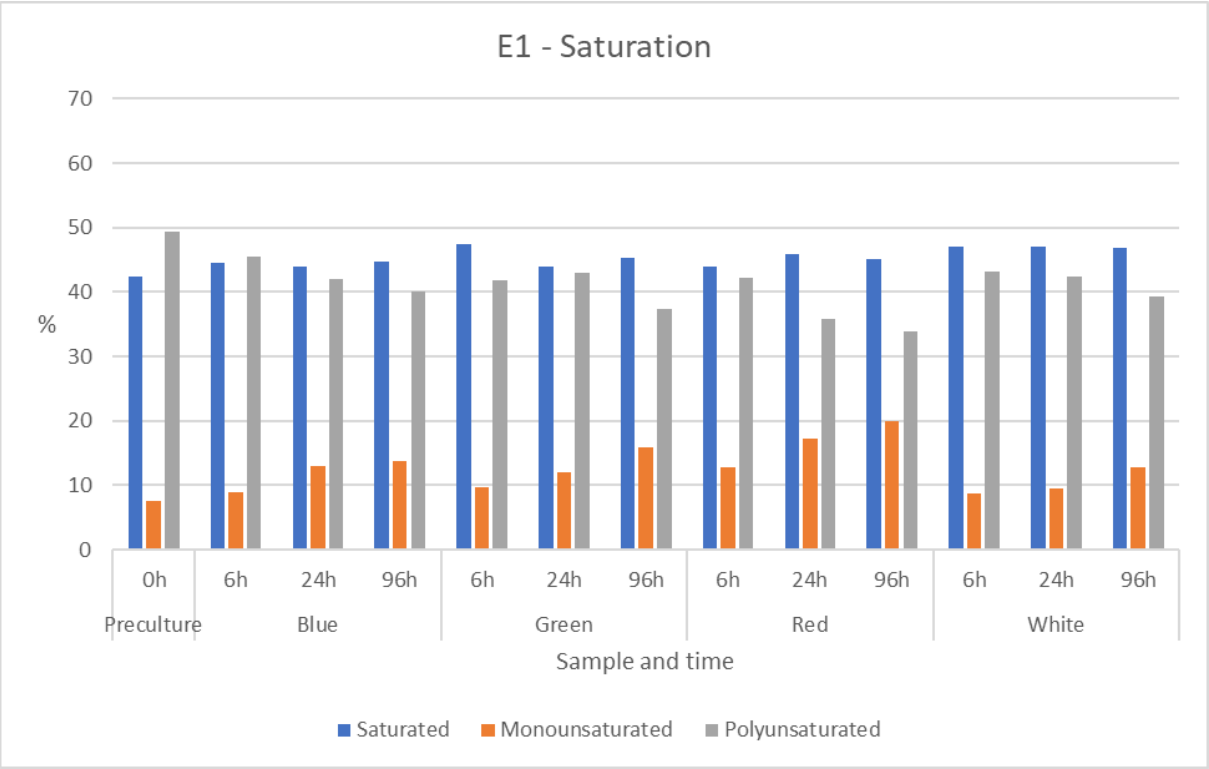

Figure 23: Saturation profile of experiment E1 (low intensities at 30 °C) differentiated into saturated, monounsaturated and polyunsaturated fatty acids for the preculture, the blue, green and red colour group and the white light control group at the time points of 6, 24 and 96 hours after start of cultivation.

The amount of SFAs increased in E% (high intensities, 30°C), before decreasing again after 96 hours and reaching a level similar to the preculture (44.6 % for preculture and on average 43.6 % (0.8 %) for colour groups after 96 hours) (Figure 24). The amount of MUFAs increased for all colours compared to the preculture, but most pronounced in the red colour group (8.1 % to an average of 12.8 % (2.7 %) with red having 17.4 %). Except for the red colour group, there was a minimum point reached at 24 hours. The amount of PUFAs decreased, and the most pronounced decrease could be found in the red colour group (46.2 % in the preculture to an average of 42.5 % (3.3 %) and only 36.9 % in the red colour group). Colour did not cause significant differences between saturated fatty acids ($p_{colour} = 0.5$), but time did ($p_{time} = 0.0006$). For MUFAs there was an effect of both colour ($p_{colour} = 0.002$) and time ($p_{time} = 0.0003$), while for PUFAs, only time affected the differences between the mean values ($p_{colour} = 0.1$. $p_{time} = 0.005$).

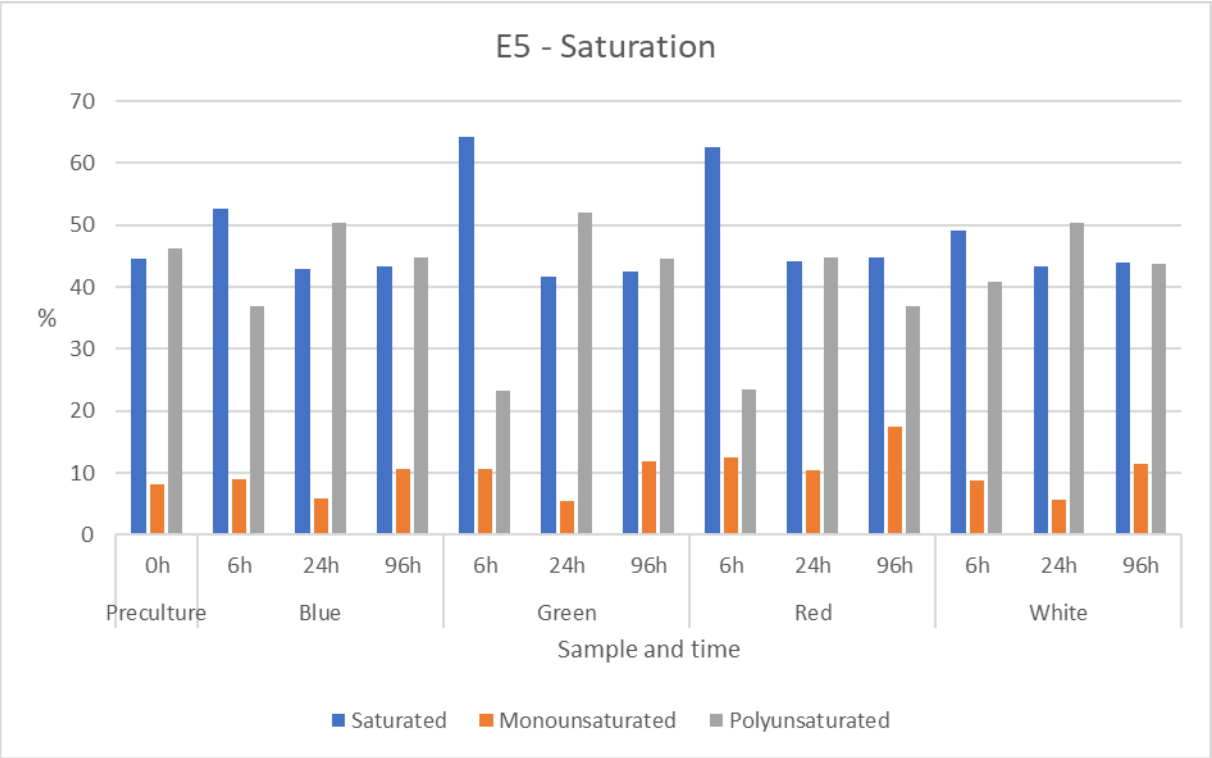

Figure 24 Saturation profile of experiment E5 (high intensities at 30 °C) differentiated into saturated, monounsaturated and polyunsaturated fatty acids for the preculture, the blue, green and red colour group and the white light control group at the time points of 6, 24 and 96 hours after start of cultivation.

5.2.1 Red light effect and converse reactions

Although all experiments gave clear trends in their changes in saturation profiles after the measuring point four days after start of cultivation, each of them showed converse reactions, except for E1 (low intensities, 30 °C). This means that if the general trend of a saturation group was to increase over time, there first was a decrease after six hours of cultivation, which sometimes continued to the 24 hour measuring point as well. If the general trend in a saturation group was to decrease over time, an increase could be observed first. Even though the p-values showed no influence of colour on the means of the saturation groups, these converse reactions were not visible for the red light group. In E2, E4 and E5 the general trend for unsaturated fatty acids was to increase. While all other colour groups first showed a decrease and only increased later, the values for the red colour group were increasing continuously. The red colour group always had the highest percentage of MUFAs compared

to the other colour groups. In E3 (moderate intensities, 20 °C) the amount of MUFAs was decreasing for all other colour groups, while the value of the red colour group stayed high. Except for this experiment, red also always had the lowest amount of PUFAs.

5.2.2 Fatty acid profiles of each experiment

The FA profiles for each experiment are described in detail, comparing the amount of each detected fatty acid between colour groups, as well as the development of each detected FA in a colour group over the time of 96 hours.

5.2.2.1 Profile for E1 (Low intensities, 30 °C)

For experiment E1 (120 µmol m^{-2} s^{-1} at 30 °C), the red light colour group was chosen as representative result to show the development of FA profiles over time. Graphs for the development of the green, blue and white light group can be found in the appendix (10.4 Fatty acid profile graphs). In figure 25, clear trends for each FA can be seen. The most important FAs were 16:0 (increase from ~ 40 % to ~ 45 %), 18:3 (decrease from ~ 25 % to ~ 13 %) and 18:1 (increase from ~ 5 % to ~ 15 %). The changes were the similar for the other colours, most evident in the red colour group. In the other colour groups, the 18:3 FA only decreased to ~ 17 % and the 18:1 fatty acid only increased to around 12 %.

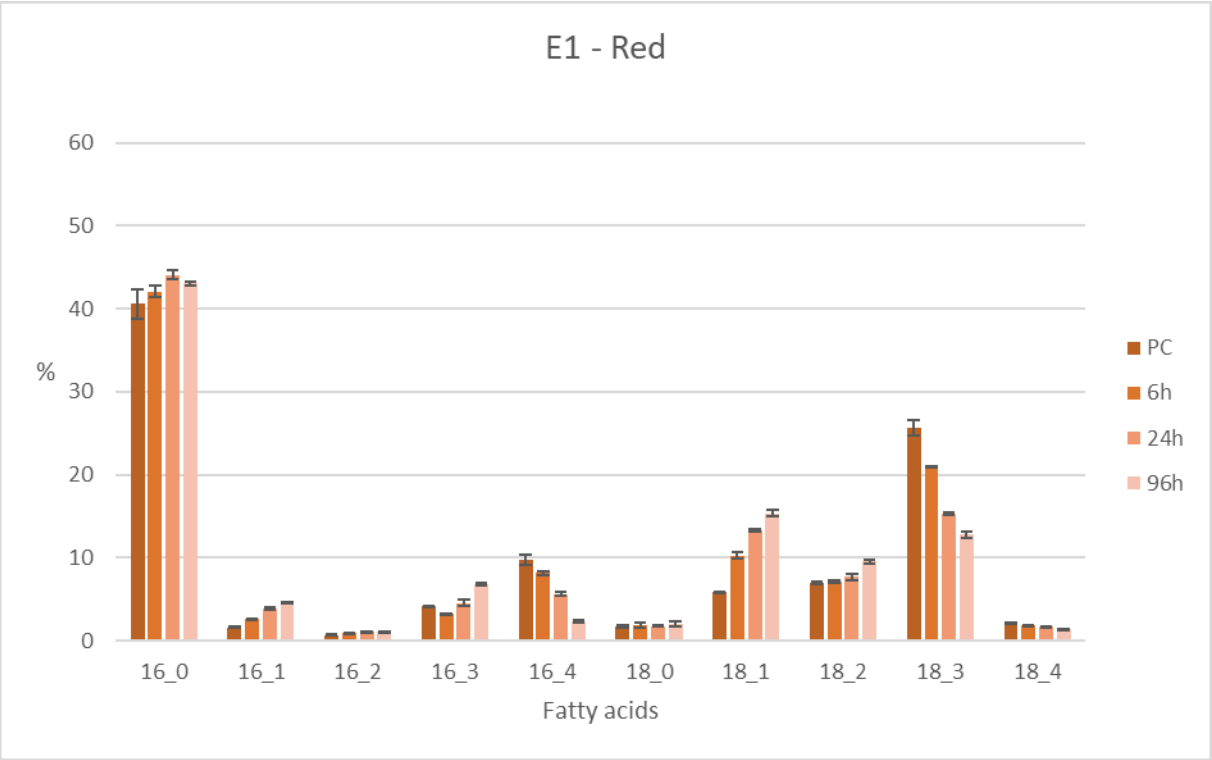

Figure 25: Development of the fatty acid composition of the red light group in experiment E1 (low intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

A comparison of the FA composition of the colour groups and the white light control group is shown six hours after the start of the experiment and compared to the fatty acid profile of the preculture (Figure 26). Cultivation under changed temperature and light condition compared to the preculture only led to small changes in the FA profile of the algae. One exception was the 18:1 fatty acid, where the red colour group had a higher value than the preculture and the other colour groups. Variations in the amount of the main FAs 16:0 and 18:3 were high between colour groups. Figure 27 shows the differences between the colour groups after four days of cultivation. Compared to the other colour groups, red had a significantly lower percentage of the 16:4 fatty acid than the blue ($p = 0.01$) and green ($p = 0.03$) colour group. Moreover, the red colour group had significantly higher percentages of the 18:1 fatty acid than the blue and green colour group ($p = 0.02$ and 0.007, respectively).

The amount of the 18:3 fatty acid in red was only significantly lower than that of the blue colour group ($p = 0.02$).

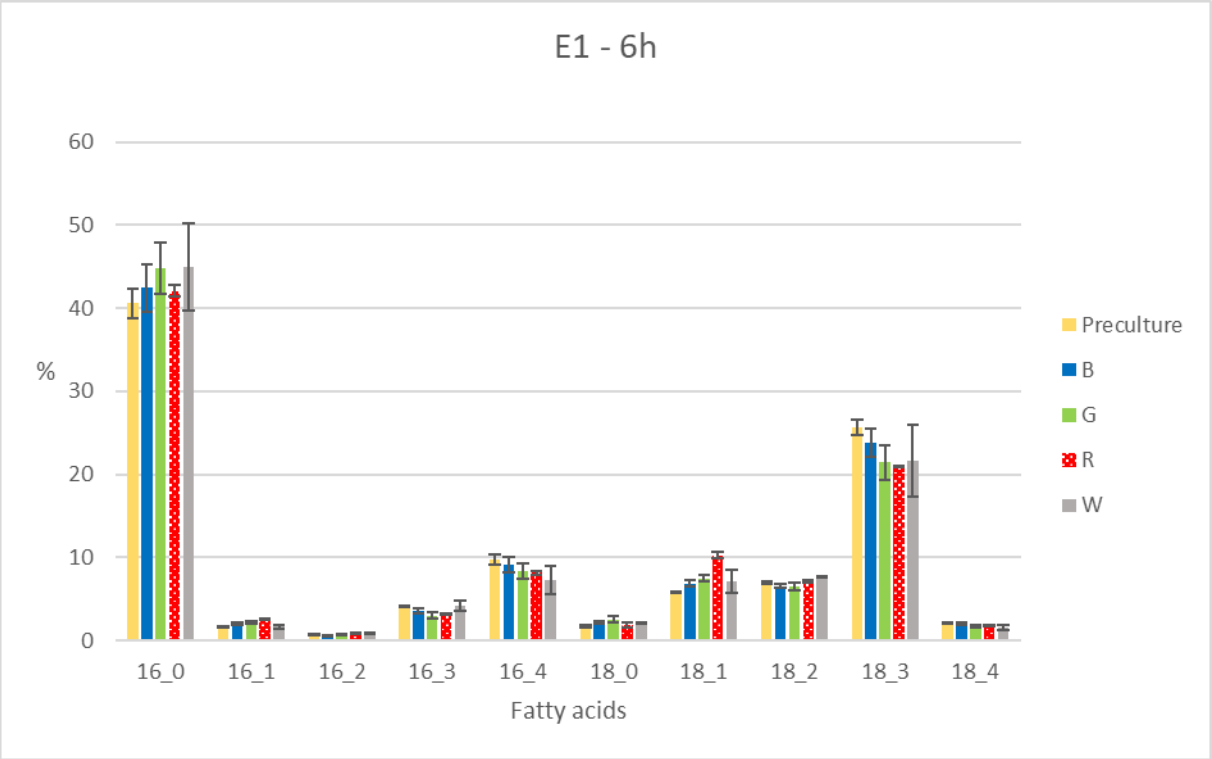

Figure 26: Comparison of the fatty acid composition of experiment E1 (low intensities, 30 °C) between the preculture, the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of six hours after start of cultivation for colour groups and the white light control group, including standard deviation bars

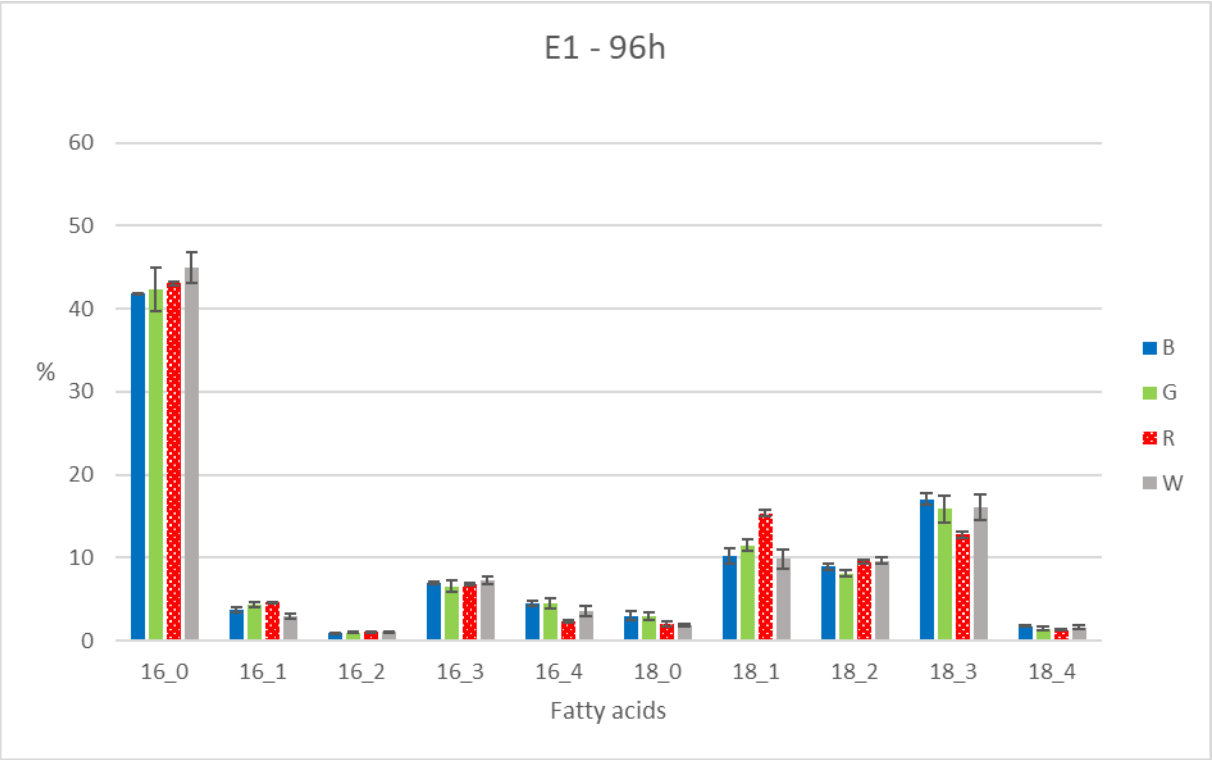

Figure 27:Comparison of the fatty acid composition of experiment E1 (low intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 96 hours after start of cultivation, including standard deviation bars.

5.2.2.2 Profile E2 (Moderate intensities, 30°)

E2 was conducted at the same temperature as the previous experiment, however the light intensities were higher. The graph for the white light control group is shown, as it is representative for the time development of fatty acid profiles of the blue and red colour group as well (Figure 28). Additionally, a graph for the green colour group is shown, as this group had some particularities in the fatty acid profile development (Figure 29). The main fatty acids 16:0 and 18:3 increased (~ 40 % to ~ 45 %) and decreased (~ 20 % to ~15 %), respectively. However, changes were not as clearly as for E1, which was due to converse reactions happening at the six hour time point. Except for 16:0, all fatty acids that were increasing in E1 were decreasing first before increasing again (16:1, 16:3, 18:1, 18:2). The two fatty acids that were decreasing (16:4 and 18:3) had similar percentages compared to the preculture after six hours and after that, started to decrease. The FA time profile of the

green colour group was different compared to the other groups. The 16:0 FA increased from ~ 40 % in the preculture to ~ 55 % after six hours. It decreased again afterwards to around 42 % at 24 hours and remained relatively unchanged until day four. The other main fatty acid, 18:3, showed a high degree of variation in the green colour group. Similar changes could be observed for the other highly unsaturated FA 16:4.

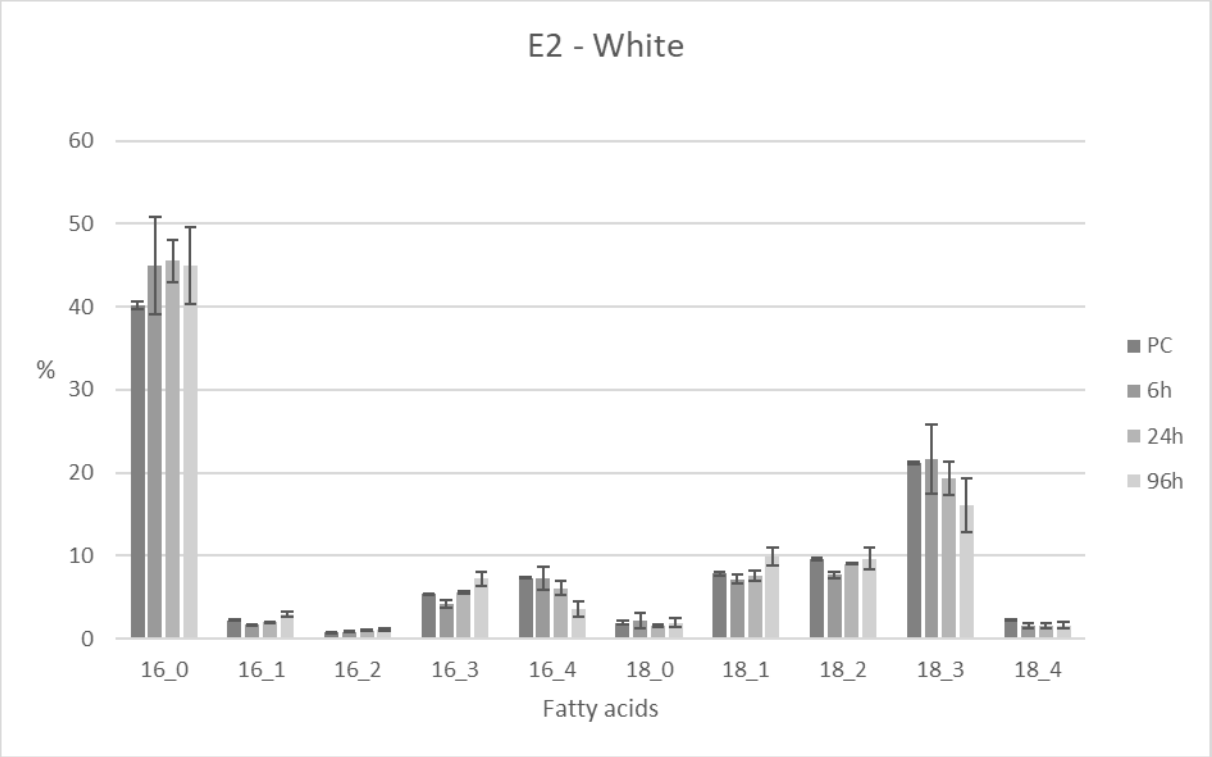

Figure 28: Development of the fatty acid composition of the white light control group in experiment E2 (moderate intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

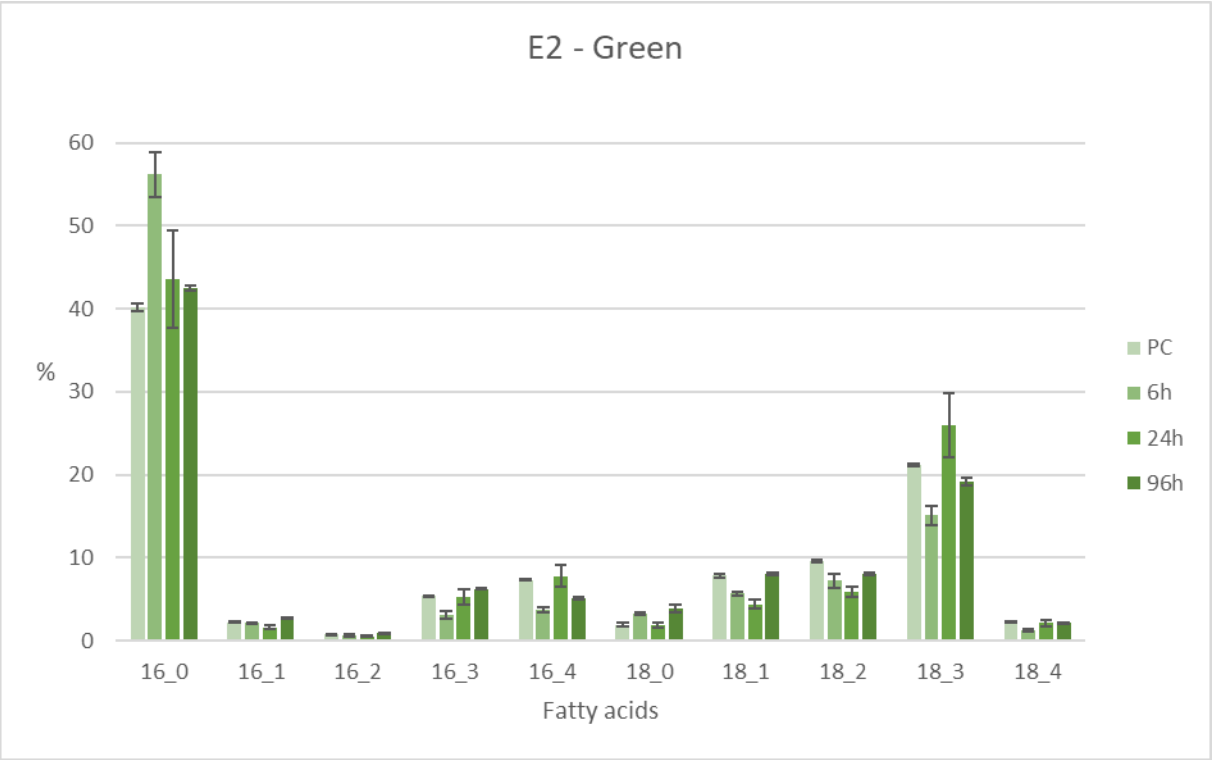

Figure 29: Development of the fatty acid composition of the green light group in experiment E2 (moderate intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

The variation between the colour groups in experiment E2 after six hours of cultivation was high (Figure 30). The amount of 16:0 was higher in the green colour group, while its amount of the 18:3 FA was lower than in the preculture, the other colour groups and the white light control group. The red colour group had higher values for the 18:1 and 18:2 fatty acids than the other colour groups, while the blue colour group had a higher percentage of the 16:4 FA than the other colour groups. 96 hours after the start of the experiment, the percentage of the 16:0 fatty acid was highest in the white light group and significantly lowest in the green colour group (~ 52 % and ~ 43 % respectively, see figure 31). For the 18:3 fatty acid, the blue colour group had a significantly higher value than the red light group ($p = 0.003$). The amount of 18:1 and 18:2 was significantly increased in the red colour group, while the amount of the 16:4 fatty acid was significantly different for all colour groups and lowest in the red colour group (for p-values see appendix, 10.5.3 Significance table fatty acids).

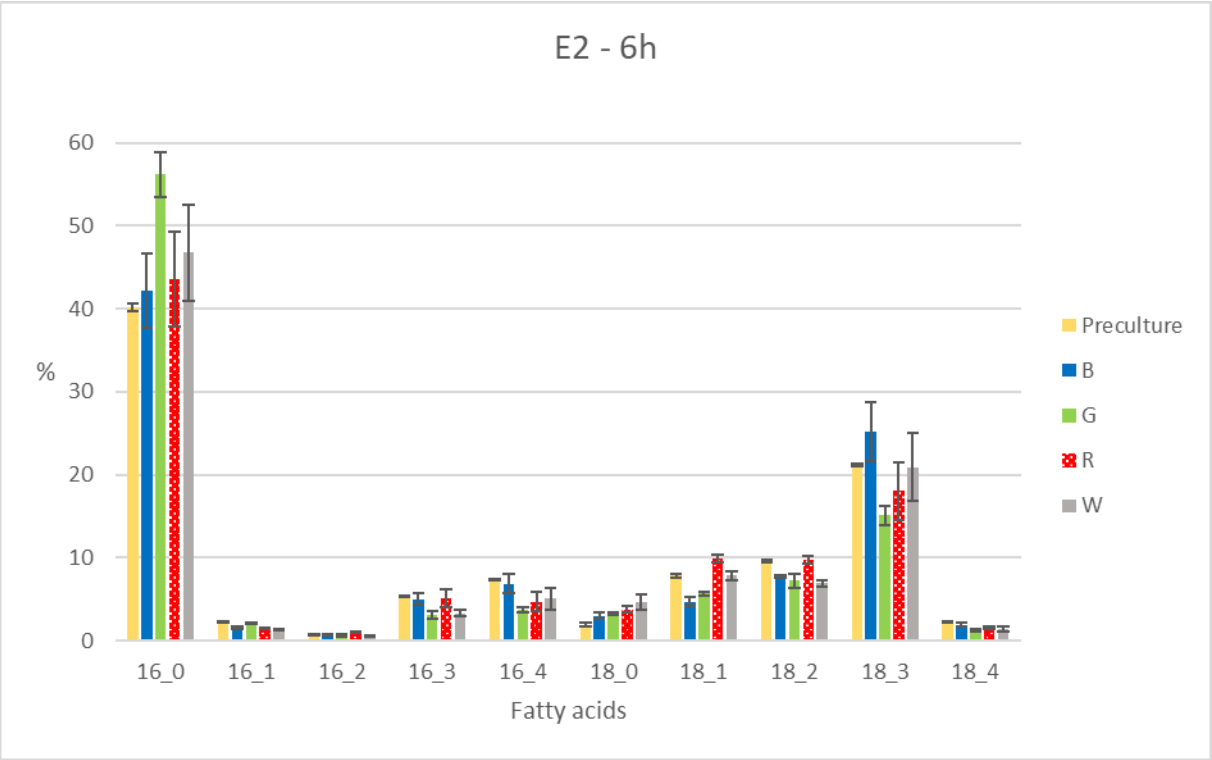

Figure 30: Comparison of the fatty acid composition of experiment E2 (moderate intensities, 30 °C) between the preculture, the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of six hours after start of cultivation for colour groups and the white light control group, including standard deviation bars.

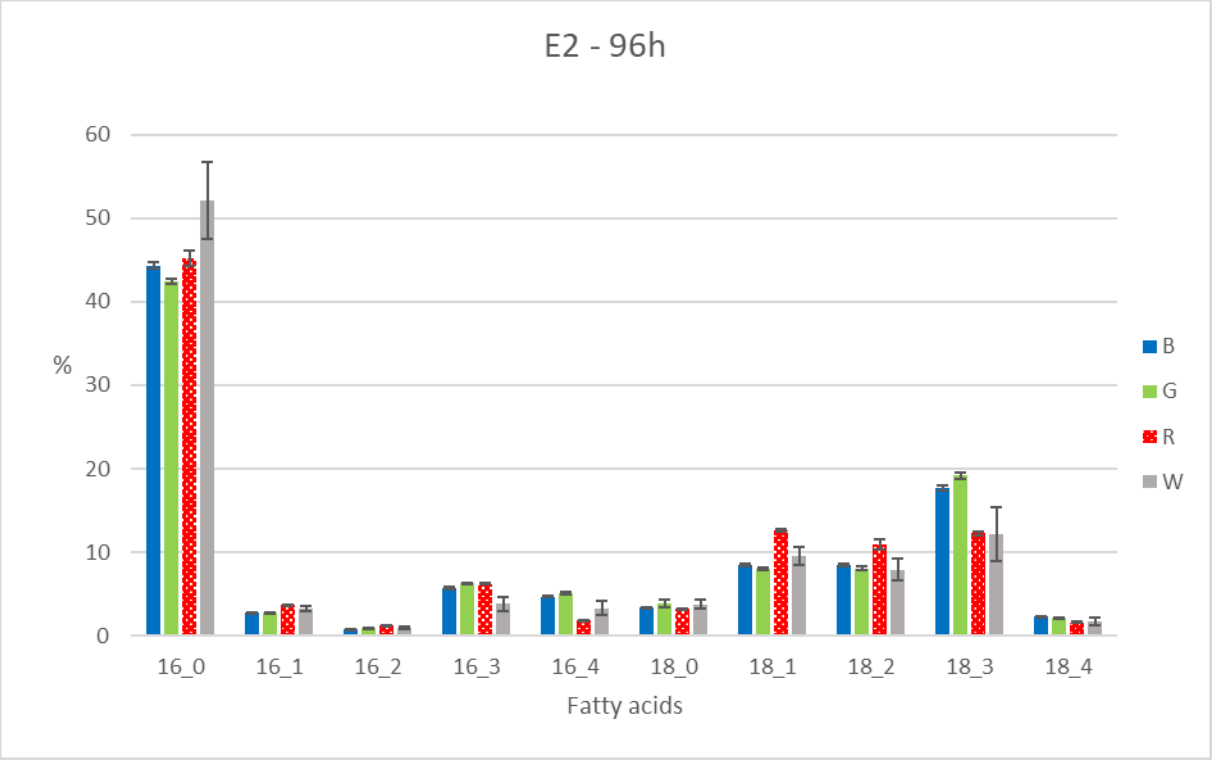

Figure 31: Comparison of the fatty acid composition of experiment E2 (moderate intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 96 hours after start of cultivation, including standard deviation bars.

5.2.2.3 Profile E3 (Moderate intensities, 20 °C)

While the experiments E1 and E2 were conducted at the temperature optimum of A. obliquus at 30 °C, E3 was conducted under moderate intensities at 20 °C. Again, the development of the fatty acid profiles overall gave the same trends for all colour groups and the green colour group was chosen as representative group for demonstration of changes in the fatty acid profiles. Figure 32 shows trends for changes in the composition, where a converse reaction can be seen at the six-hour time point. PUFAs showed a decrease, while saturated and monounsaturated fatty acids showed an increase. Exceptions from this were FAs that were present in low amounts (16:1, 16:2). At 24 hours, PUFAs increased again, while SFAs and MUFAs showed a decrease. The main fatty acid 16:0 decreased slightly, while the 18:3 fatty acid showed an increase from ~ 22 % in the preculture to ~ 33 % after 96 hours. The PUFAs 16:3, 16:4 and 18:4 increased, with 16:4 reaching ~ 12 % at day four, while 16:1, 16:2, 18:0, 18:1 and 18:2 decreased. While the percentages of the blue and white colour group looked similar to the ones of the green colour group, the red colour group had a lower increase in PUFAs. Here the 16:4 fatty acid only reached around 8 % and the 18:3 fatty acid was at ~ 23 % at day four, which was 10 % less than the value for the green, blue and white colour group.

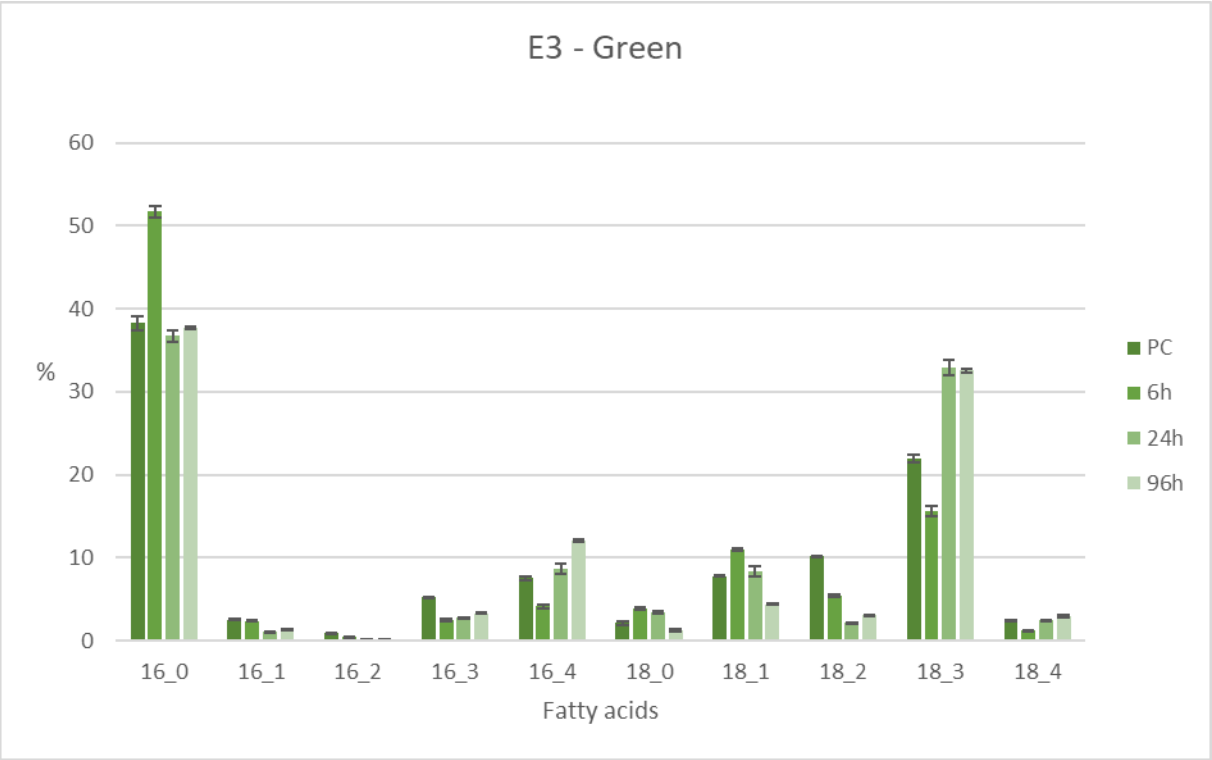

Figure 32: Development of the fatty acid composition of the green light group in experiment E3 (moderate intensities at 20 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

Figure 33 shows that the development of fatty acids compared to the preculture was similar for all colour groups in E3. The percentages of the 16:0 fatty acid showed an increase of ~ 14 % compared to the preculture, for the 18:0 and 18:1 FA the colour groups also had an increased value. Compared to the preculture, the colour groups had lower amounts of the 16:3, 16:4, 18:2, 18:3 and 18:4 fatty acids. The comparison of colours at 96 hours after the start of the experiment showed comparable values for the green, blue and white colour group (Figure 34).

Statistical analysis shows that the amount of all tested FAs is significantly different between the blue and green colour group. In the red colour group one of the triplicates was lost, therefore no statements about statistical differences could be made. Red had higher amounts of the 16:3, 18:1 and 18:2 fatty acids, while the amounts for 16:4 and 18:3 were lower than in the other colour groups and the white light control groups.

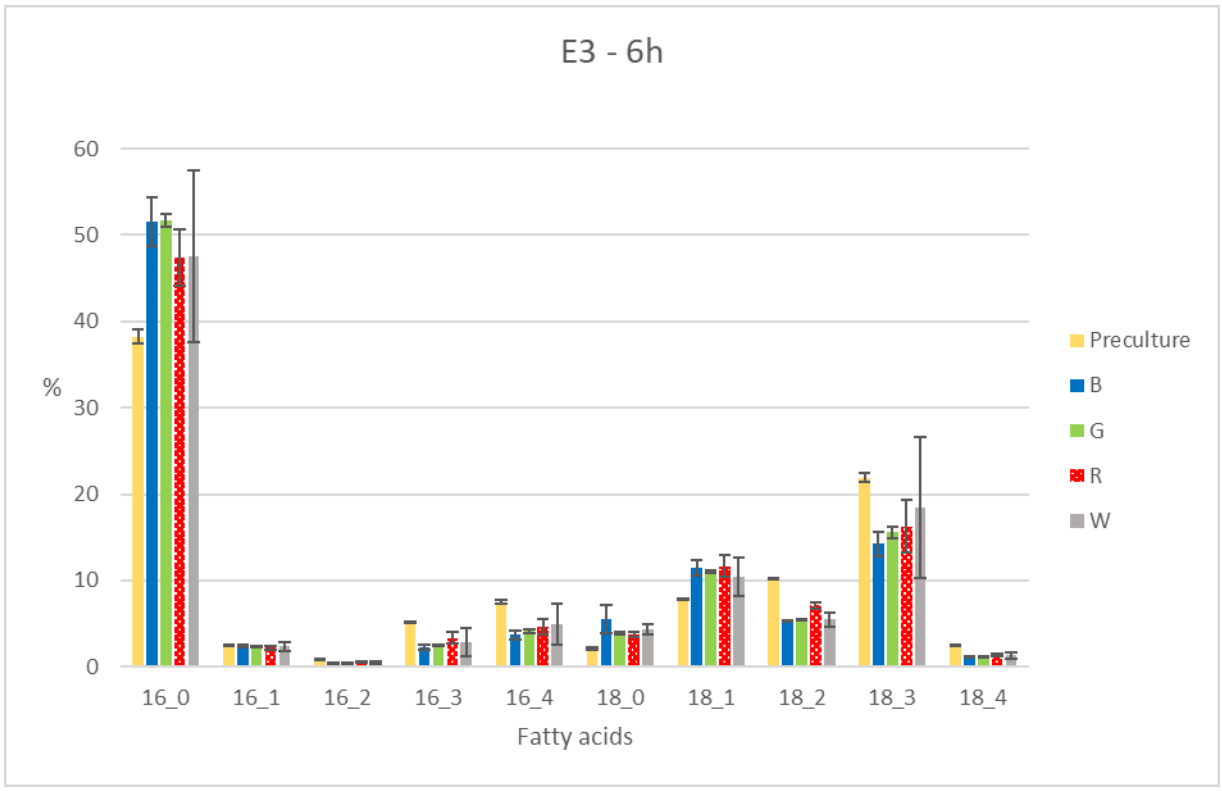

Figure 33: Comparison of the fatty acid composition of experiment E3 (moderate intensities, 20 °C) between the preculture, the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of six hours after start of cultivation for colour groups and the white light control group, including standard deviation bars.

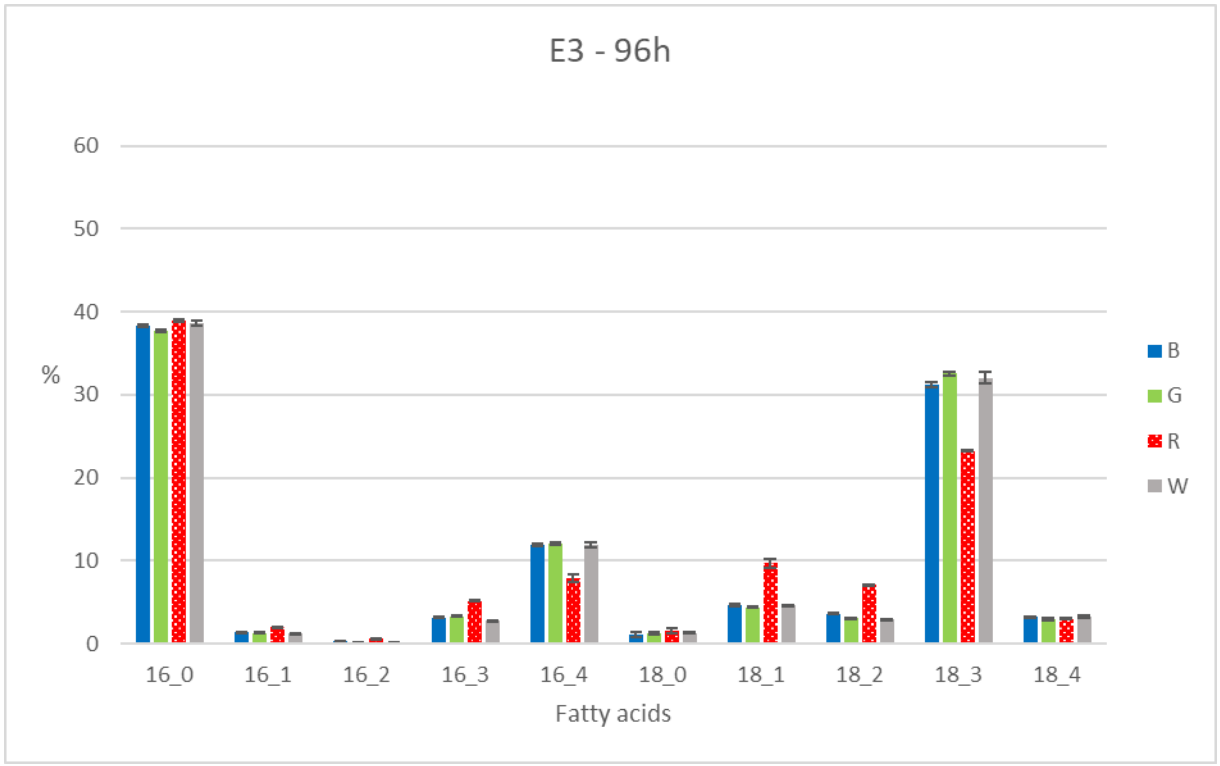

Figure 34: Comparison of the fatty acid composition of experiment E3 (moderate intensities, 20 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 96 hours after start of cultivation, including standard deviation bars.

5.2.2.4 Profile E4 (Moderate intensities, 35 °C)

E4 was conducted above the optimum temperature of *A. obliquus* at 35 °C under moderate light intensities. Changes in the FA profiles were the same for all colour groups, the white light control group was chosen as representative graph (Figure 35). The main FA 16:0 increased from ~ 39 % to ~ 47 %, while 18:3 decreased from ~ 21 % to ~ 17 %. 16:1, 16:4, 18:0 and 18:4 decreased, while 18:1 and 18:2 increased from six hours until 96 hours after the start of the experiment.

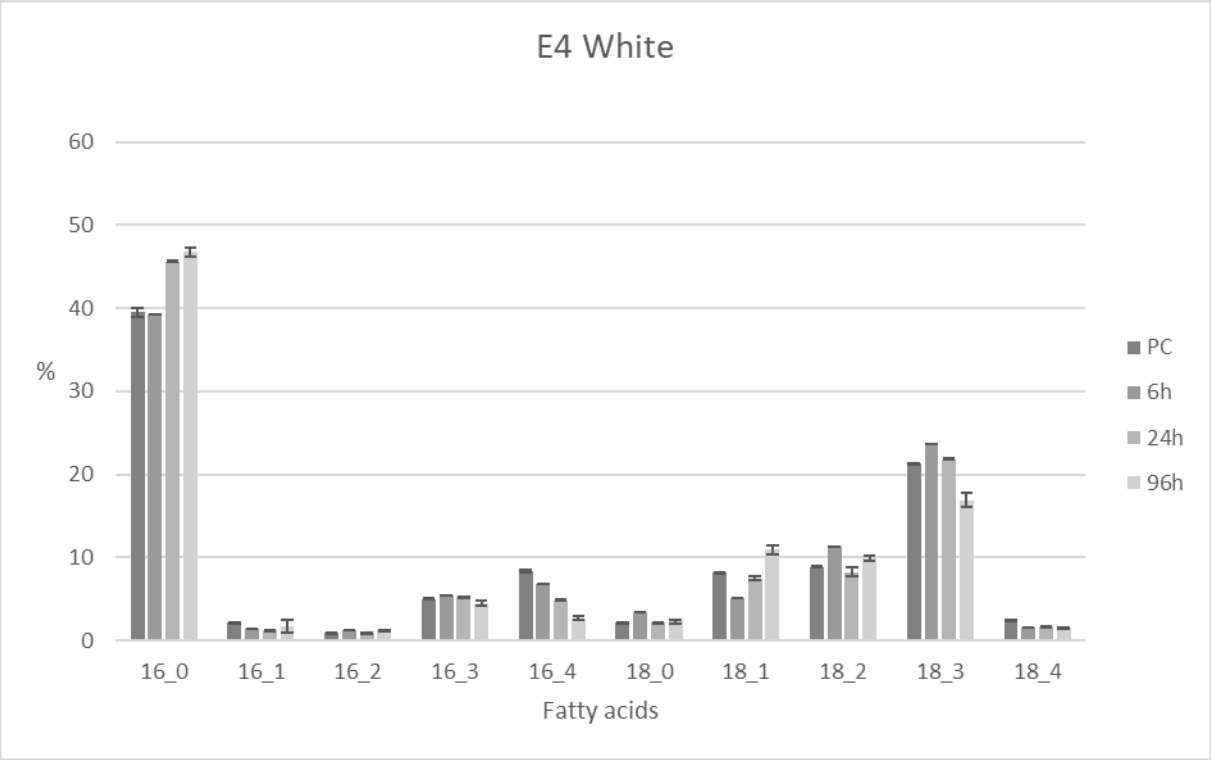

Figure 35: *Development of the fatty acid composition of the white light control group in experiment E4 (moderate intensities at 35 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.*

Experiment E4 gave a weaker converse reaction compared to other experiments (Figure 36). The colour groups and white light control group had higher values of the 18:0 and 18:2 FAs than the preculture, while their percentages for the 16:4 and 18:1 fatty acids were lower, except for the red colour group, which had a similar percentage of 18:1 like the preculture. There were no changes for the 16:0, 16:1 and 16:3 fatty acids after six hours of cultivation.

After four days, the fatty acid profiles of the four colour groups were similar (Figure 37). The green colour group had lower amounts of the 16:0 and 18:2 fatty acids than the other colour groups (significant, p-values see appendix, 10.5.3 Significance table fatty acids).

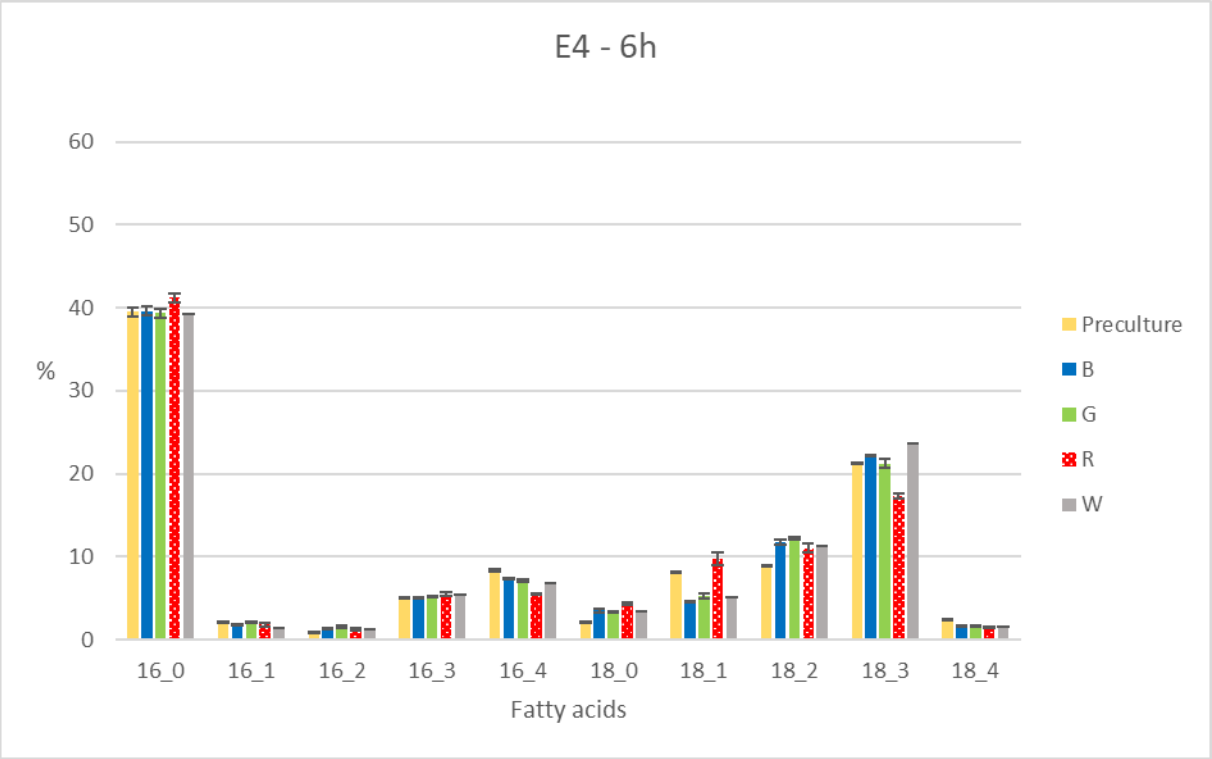

Figure 36: Comparison of the fatty acid composition of experiment E4 (moderate intensities, 35 °C) between the preculture, the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of six hours after start of cultivation for colour groups and the white light control group, including standard deviation bars.

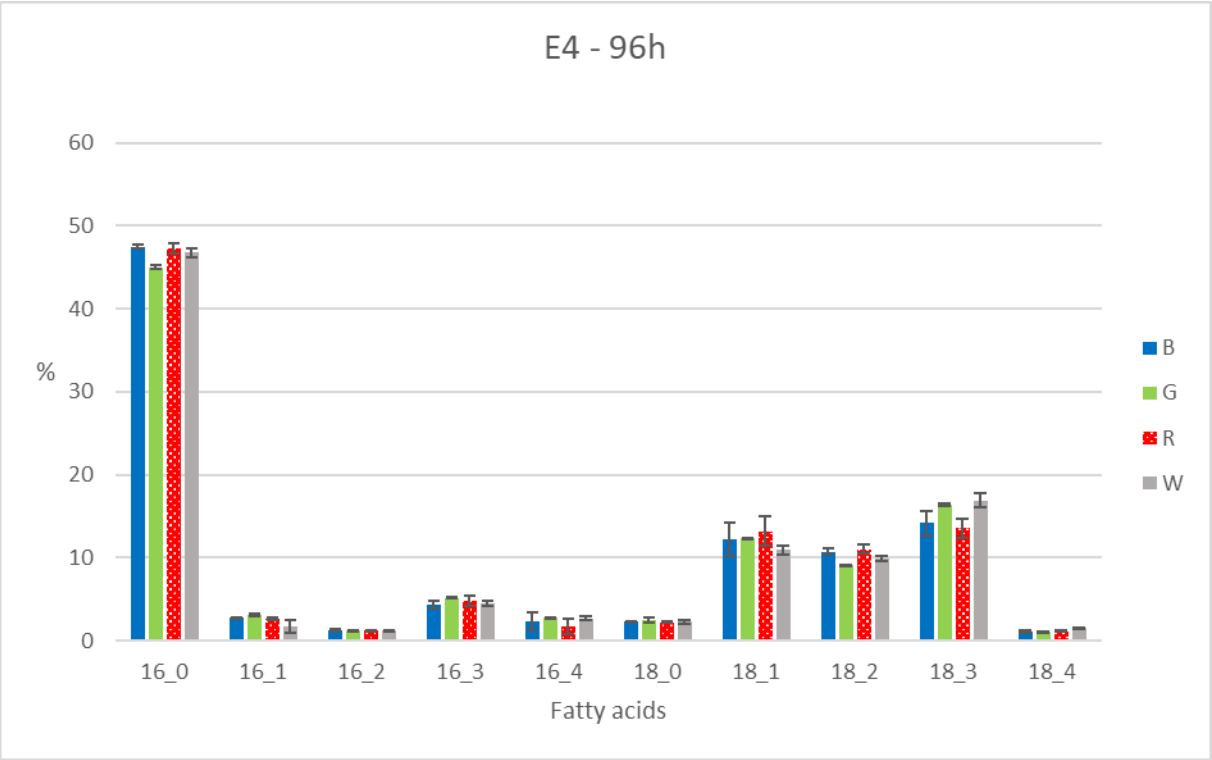

Figure 37:Comparison of the fatty acid composition of experiment E4 (moderate intensities, 35 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 96 hours after start of cultivation, including standard deviation bars.

5.2.2.5 Profiles E5 (High intensities, 30 °C)

The experiment E5 was conducted at the optimum temperature for A. obliquus (30 °C) at an intensity of 800 µmol m^{-2} s^{-1}. All colour groups showed the same trends. The blue, green and red colour group showed a decrease in PUFAs and increase in SFAs at the six-hour time point, this was less pronounced in the white light control group. The trends in the white light group are described first (Figure 38). The amount of the main fatty acid 16:0 stayed at a value of ~ 41 %, while the percentages of the 18:3 fatty acid showed a decreasing trend after 96 hours. Other PUFAs decreased as well (16:4, 18:4), while the 16:3 fatty acid increased. Changes over time were more pronounced in the colour groups than in the white light control group and especially visible in the red colour group (Figure 39) There was a strong decrease in PUFAs together with a high increase in the SFA 16:0, which increased to ~ 57 %. The 18:3 fatty acid had a minimum value of ~ 10 % at the six hour time point in the red colour group.

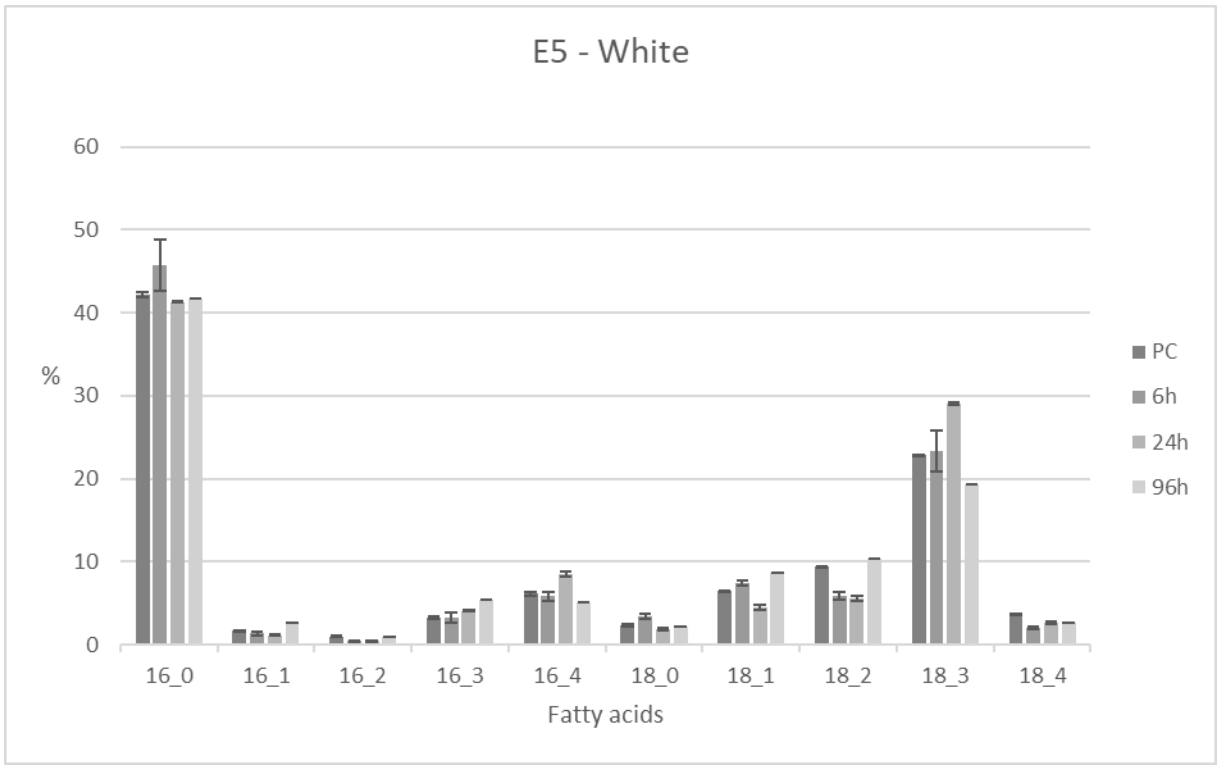

Figure 38: Development of the fatty acid composition of the white light control group in experiment E5 (high intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

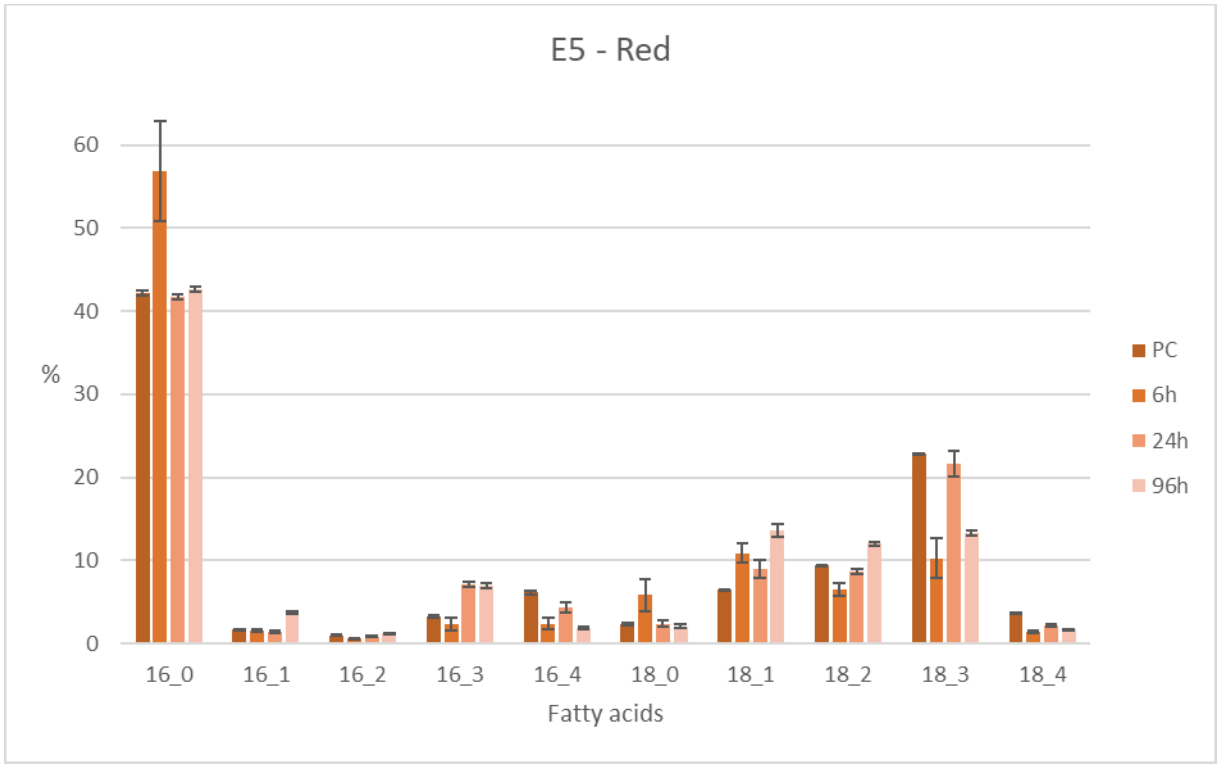

Figure 39: Development of the fatty acid composition of the red light group in experiment E5 (high intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

The differences between the preculture and the colour groups after six hours of cultivation were high, while the fatty acid composition of the white light group was similar to that of the preculture (Figure 40) The red and green colour groups had higher values for the 16:0 and 18:0 SFAs, while their values for 16:3, 16:4 and 18:3 PUFAs were lower. For the 18:2 and 18:4 fatty acids, all colour groups had lower percentages than the preculture. Four days after the start of the experiment, the percentages of the colour groups were much more alike (Figure 41). The values for the 16:4 fatty acid were significantly lower in the red colour group, while the percentages of the 16:0, 18:1 and 18:2 fatty acids were significantly higher in the red than in the blue and green colour group (p-values see appendix, 10.5.3 Significance table fatty acids).

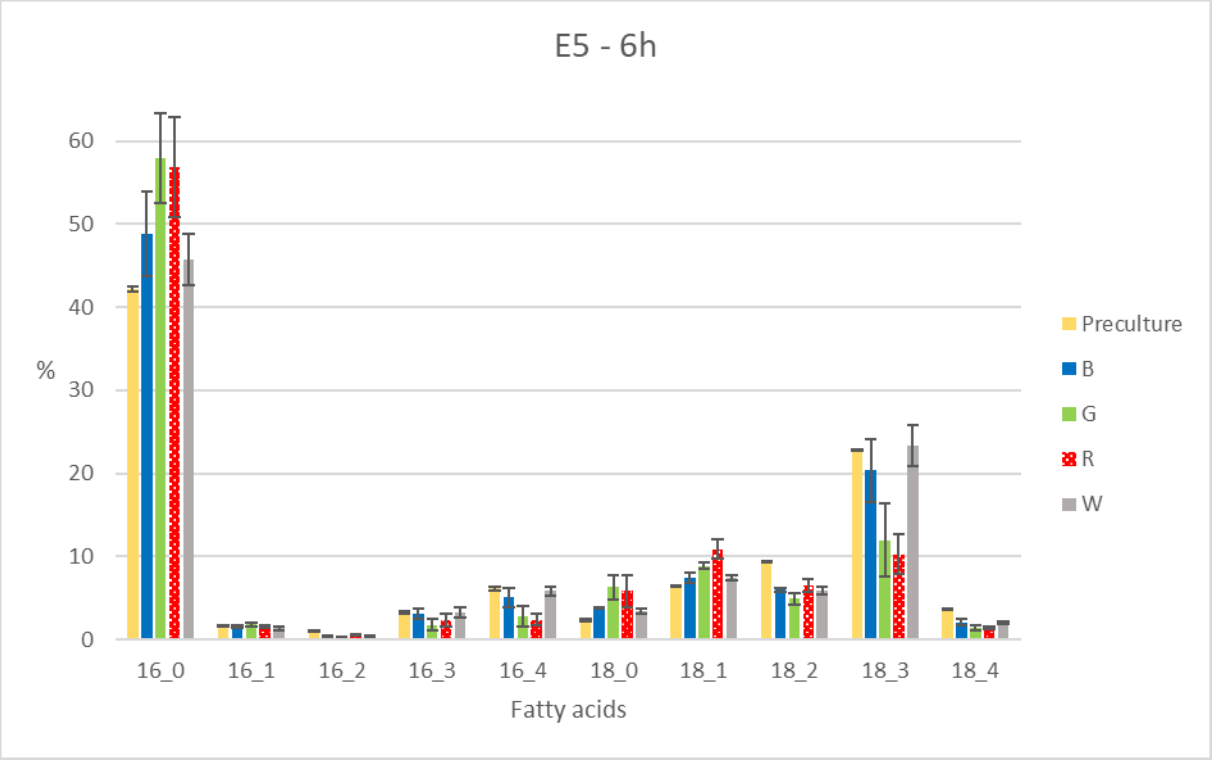

Figure 40: Comparison of the fatty acid composition of experiment E5 (high intensities, 30 °C) between the preculture, the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of six hours after start of cultivation for colour groups and the white light control group, including standard deviation bars.

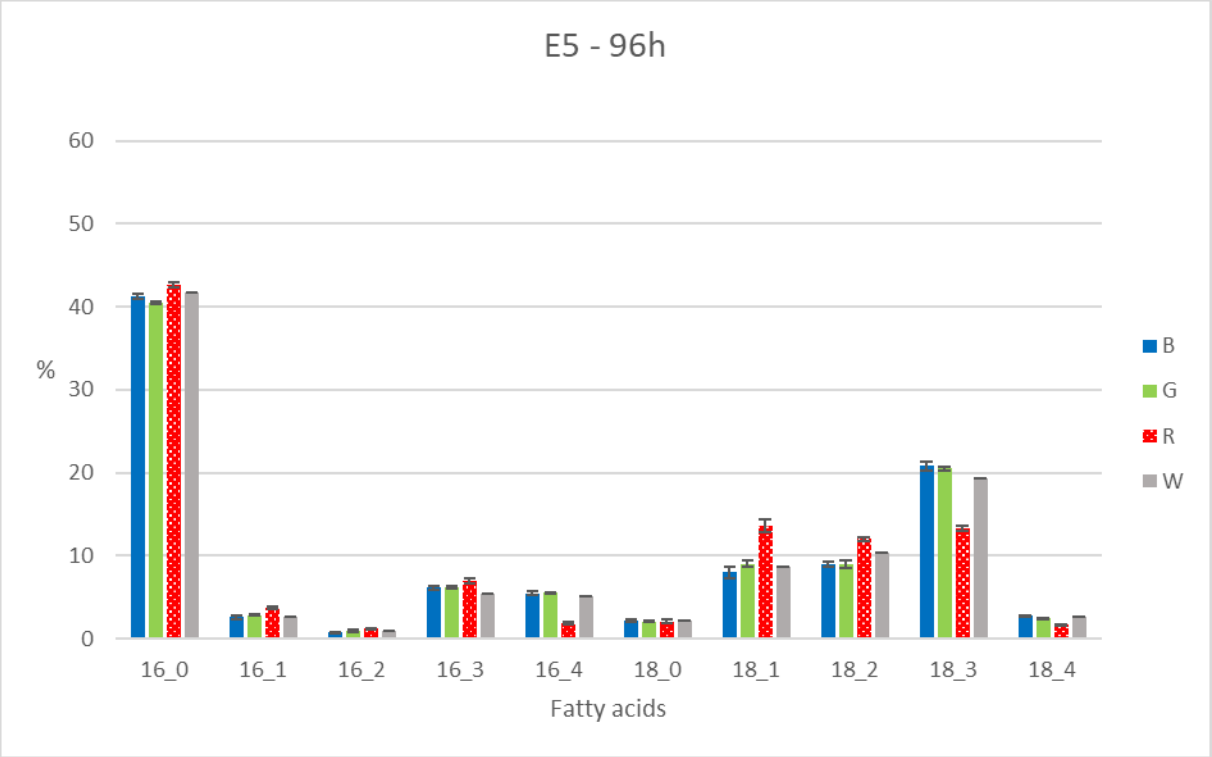

Figure 41: Comparison of the fatty acid composition of experiment E5 (high intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 96 hours after start of cultivation, including standard deviation bars.

5.2.3 Summary of percentages of fatty acids for all experiments at 96 hours

A final summary for differences between the fatty acid profiles of all experiments is given. As there was a pronounced variation after six and 24 hours of cultivation, the differences four days after the start of the experiment are described. The table with values for this comparison can be found in the appendix (10.4.6 Fatty acid profile comparison for all experiments after 96 hours).

Overall E3, which was conducted at moderate intensities at 20 °C, had the highest amount of PUFAs. The amount of the 16:4 fatty acid was with ~ 12 % more than double as high as in the other experiments (E1 and E2 ~ 4 %, E4 ~ 2.5 % and E5 ~5 %). In all five experiments, the red colour group had a significantly lower amount of the 16:4 fatty acid. For the 18:3 fatty acid, E3 had a maximum value of ~ 32 % (red only ~ 23 %), while E1, E2 and E4 all

had around 15 % to 18 % and E5 had ~ 20 %. E3 also has the highest amount of the 18:4 fatty acid with ~ 3 %. This was the only highly unsaturated fatty acid where the red light colour group did not always have values that were significantly lower than they were for the other colour groups. While having the maximum values for polyunsaturated fatty acids, E3 also had the lowest amount of 16:0 (~ 38 % compared to 41 % to 47 % in the other experiments) and the lowest amount of 18:0 (~ 1.3 %) fatty acid. 16:1, 16:2 and 16:3 as well as 18:1 and 18:2 were lower in E3 than in all other experiments, with red always having higher values than the blue, green and white colour group.

Both E2 (Moderate intensities, 30 °C) and E4 (Moderate intensities, 35 °C) had high values of saturated fatty acids. The maximum value for the main fatty acid 16:0 was reached in E2 (30 °C) with 52 % (white colour group), however there was a high variation (green with ~ 2.5 %). E4 (35 °C) also had high values but varied between ~ 45 % and ~ 47.5 %. Concerning the 18:0 FA, the percentages were higher in E2 (~ 3.5 %) than in E4 (~ 2.2 %). For the other main fatty acid 18:3, the maximum value in E2 was higher than the one of E4, but it also had a higher variation between the colour groups.

E1 and E2, experiments conducted at 30 °C and with low and moderate intensities, respectively, had quite comparable fatty acid profiles.

E5, the experiment conducted at 30 °C and high intensities, had favourable values when looking at the amount of saturated and polyunsaturated fatty acids. The percentages of the SFAs 16:0 (~ 41 %) and 18:0 (~2.1 %) were higher than in E3 (20 °C), but still low compared to the other experiments (E1, E2 and E4). With ~ 6 % the amount of 16:3 was as high as in E1 and E2 and higher than in E4. The same was the case for the 16:4 fatty acid (~ 5.5 %, with red having ~1.8 %). E5 also had the second highest values for 18:3 and 18:4 after E3, with ~ 20 % and 2.5 % respectively. In general, the relative fatty acid levels of the red colour group varied significantly compared to the blue, green and white light group,

which were quite similar. The general trend was that the red light group had a higher amount of SFAs and a lower amount of PUFAs.

6 Discussion

6.1 Growth and photosynthetic activity

Increasing light intensities stimulated growth and a cultivation temperature of 30 °C gave a higher increase in biomass than 35 °C, while 20 °C gave the lowest growth. This is in accordance with previous findings showing that the temperature optimum for growth of *A. obliquus* is at 30 °C (Hindersin, 2013). The control group with full spectral light had the best growth results, which is presumably due to the fact that this is the spectrum algae are adapted to. Between the colour groups, red light gave the highest increase in biomass, while blue and green light gave lower growth results. At low intensities, blue and green light performed similar. Increasing intensities gave increasing growth under green light, with the green light group performing better than the blue light group at moderate intensities and the green light group performing better than the red light group at high intensities. Here it even reached similar biomass values as the white light control group. Blue light always gave the lowest growth, independent of light intensity or cultivation temperature used, which is in accordance with the results of previous studies (Ooms et al., 2017). A reason for that could be that blue spectral light has a higher energy content than required for photosynthesis. Plant cells therefore accumulate photoprotective pigments to prevent damage by reactive oxygen species and the energy is dissipated as heat instead of being used for photosynthesis (Schulze et al., 2014).

It could not be determined from this experiment, whether the differences between colour groups were solely caused by intensity or by the intensity-correlated biomass density of the cultures. Biomass values for the green and blue colour group were alike for the experiment at low intensities, however here only dry weights of around 1 g L^{-1} were reached in the end of the experiment. For experiments with higher intensities, the green light group had higher growth rates than the blue light group shortly after passing dry weights of 1 g L^{-1}. While the

red light group in general reached higher algae dry weights, they were not significantly higher than the values for the green light group at the end of the experiments with moderate intensities, where dry weights of around 3.5 g L^{-1} were reached. In the experiment using high intensities, the green light group started having an increased growth compared to the red light group above a biomass value of 3.5 g L^{-1}. To distinguish between the effects caused by spectral light and culture density, future experiments could be performed that either include a longer cultivation period or start with higher biomass concentrations. Nonetheless, this study gave similar results to previous studies concerning cultivation of microalgae under different spectral light and light intensity conditions. In a range of low to moderate intensities, white and red light performed better than green and blue light. This is in accordance with previous experiments, where higher biomasses for the cultivation of algae under white and red light compared to blue and green light were observed in *Chlorella vulgaris* (Hultberg et al., 2014a). With 100 μmol m^{-2} s^{-1} the intensities used in that study were lower than the lowest intensity used in this study. Besides having a high growth at intensities of 800 μmol m^{-2} s^{-1}, the green light group also had the highest effective PSII quantum yield (Y(II)). This indicates that algae exposed to green light were able to use the light more efficiently than the other colour groups. In another study, red light similarly stimulated growth in microalgae at low light intensities, while green light gave better growth results for high light intensities (Ooms et al., 2017). This was explained by the low absorbance of green light, which leads to light dilution in the microalgae containing photobioreactor (Ooms et al., 2017). At low densities, this is a disadvantage, as most of the low absorbed green light is lost in contrast to red light, which is absorbed with higher efficiency. In dense microalgae cultures, there is usually a steep light gradient, meaning the microalgae in the front layer are absorbing light, while algae in deeper layers are overshadowed. This consequently leads an imbalance with part of the algae possibly having oversaturated photosystems, while others are overshadowed and have a reduced rate of

photosynthesis. These problems can be reduced, but not eliminated by good mixing of the culture. Green light on the other hand is weakly absorbed, enabling it to deeply penetrate into the algae solution, where it is steadily absorbed without causing photoinhibition (Ooms et al., 2017). Therefore, a high content of green light in microalgae cultivation might contribute to a better and more energy efficient light use.

Another explanation for a better performance of the green light group at higher intensities would be that the low absorbance of green light by chlorophyll pigments causes the algae cells to react with a compensatory increase in chloroplasts or a re-arrangement of the thylakoid structure (Hultberg et al., 2014a). Terrestrial plants respond similarly to green light as to low light intensities. The following shade avoidance symptoms include an increase of grana stacks in the thylakoid membrane, by which the plant enhances its photosynthetic activity to be able to grow to a position where it receives more light (Zhang et al., 2011). This could explain the good performance of *A. obliquus* and other microalgae under green light, as the low absorbance leads to the same reactions as low light or shade conditions. It has been shown before that low light intensities give higher photosynthetic efficiency than high light intensities (de Mooij et al., 2016; Hindersin, 2013).

The results of this thesis add to the pool of studies disproving the established opinion that low absorbance of green light leads to low growth results. Especially in microalgae, green light seems to be used with a high efficiency, leading to good growth results (Emerson & Lewis, 1943; Kubín et al., 1983; Ooms et al., 2017). In conclusion, this is explained by light dilution in the photobioreactor and physical changes due to shade reaction in the algae cells. The best growth results in this study were found at high intensities of 800 μmol m^{-2} s^{-1} using full spectral white light or spectral green light at a cultivation temperature of 30 °C.

6.2 Fatty acid profiles

In this study, the fatty acid profiles of the microalgae *Acutodesmus obliquus* grown under different light spectra, light intensities and temperature conditions were compared. The overall suitability of *A. obliquus* for large scale production is already reckoned as high, because of its high growth rates and ability to grow in cheap growth media (Abomohra et al., 2013; Breuer et al., 2012; Hindersin et al., 2013). However, these recommendations have mainly focused on using *A. obliquus* to produce biofuels and bioenergy. Fatty acids of microalgae can also be used as food and feed additives, where they can act as valuable supplements to promote health in animals and humans (Chee et al., 2005; Davis & Kris-Etherton, 2003; Rymer & Givens, 2005). An economically valuable profile includes a high degree of polyunsaturated fatty acids, which are essential to human health and have disease-preventing properties (Riediger et al., 2009; Ruxton et al., 2004). Targeted production of valuable PUFAs requires knowledge about how lipid accumulation and fatty acid composition can be influenced, preferably without having adverse effects of a decrease in growth, which can occur when stressors are enhancing lipid accumulation (Francisco et al., 2010). The aim of this study was to investigate the impact of temperature, light spectra and light intensity on the fatty acid profile of *A. obliquus*.

Fatty acids above 1 % in *Acutodesmus obliquus* were the 16:0, 16:1, 16:2, 16:3, 16:4, 18:0, 18:1, 18:2, 18:3 and 18:4 fatty acids. The main FAs were the SFA 16:0 with around 44 % and the PUFA 18:3 with around 21 %. A previous study of the fatty acid profile of *A. obliquus* revealed similarly that the main fatty acids were the 16:0 FA and 18:3 FA, however the amount of the 16:0 FA was lower and the amount of PUFAs was higher (Abomohra et al., 2013). This difference could be explained by the lower cultivation temperatures (25 °C) used in the previous study and cycle of 16 hour light and eight hour dark period.

The amount of the 18:3 PUFA increased with increasing light intensities and decreasing temperatures. It is often present in high concentrations in photosynthetic organisms due to its high concentration in lipids associated with plant chloroplast (Erwin, 1963; Ferrari & Benson, 1961). A study by Nichols (1965) documented that the metabolism in microalgae chloroplasts seems to act similar to the metabolism in plant chloroplasts, as the content of 18:3 fatty acid in *C. vulgaris* increased with photosynthetic activity. The spectral light used had a low impact in this study, as the contents of PUFAs were similar for cultivation under green, blue and white light and only reduced in the red light group. The results are in contradiction with those of Hultberg et al. (2014b), who reported an increase in the amount of α-linolenic fatty acid (18:3) during the cultivation of *C. vulgaris* under green light. The study used low light intensities of 100 μmol m^{-2} s^{-1} together with low cultivation temperatures of 20 °C. However, similar parameters were used in the present study (E1 with 120 μmol m^{-2} s^{-1} and E3 with 20 °C) and in both cases, the green light group did not have significantly higher values for the 18:3 FA compared with the blue or white light group. The difference therefore seems to be caused by the spectral light used. The light emitting diodes used by Hultberg et al. (2014b) gave a clear differentiation of spectral areas of blue and green light, with blue light ending at 500 nm and green light starting at 500 nm. In this thesis, the transmission spectra of the filter foils overlapped, with blue ending at 540 nm and green starting at 450 nm. It could therefore be speculated, that a receptor enhancing the production of chloroplasts and therefore causing higher contents of the 18:3 FA is stimulated by the spectral area between 500 nm and 540 nm. This would explain why Hultberg et al. (2014b) only found an effect of green light, while in this study, it was found for blue light and white light containing the full spectrum as well. For multiple terrestrial plants, the signalling properties of green light have already been demonstrated and recent studies proved the influence of green light on gene expression in *Arabidopsis thaliana* (Dhingra et al., 2006;

Zhang & Folta, 2012). However, further studies are needed to support this theory for *A. obliquus*.

During the experiment conducted at a low cultivation temperature of 20 °C, a strong increase in PUFAs was observed. This increase could be due to the algae in the cultivation unit being exposed to lower temperatures than in the preculture (26 °C). The amount of PUFAs increased to ensure adequate membrane fluidity at the colder temperatures, which is generally considered an adaption mechanism to cold temperatures (Lewis, 1962). Thylakoid membranes of chloroplasts comprise the highest amount of PUFA containing lipids compared to other membranes and seem to be of a high importance for the biogenesis and maintenance for chloroplast ultrastructure (Hugly & Somerville, 1992; Kunst et al., 1989). Colder temperatures caused the amount of the 18:3 FA to increase in *A. obliquus*, while the amount of the 16:1 FA decreased. The same mechanism has been observed in *Arabidopsis thaliana*, where it contributed to low temperature fitness, which shows that general processes in plants and microalgae seem to be similar (Murphy, 1986).

For all the other experiments, where the temperature was either 30 °C or 35 °C, the amount of PUFAs decreased and the amount of SFAs increased. The acclimation to warmer environments has been shown to decrease membrane polyunsaturation (Raison et al., 1982). The differences between the fatty acid composition of *A. obliquus* at cultivation temperatures of 30 °C and 35 °C were low, which shows that it has reached a stage where no more adaptions to temperature took place.

The adaption of the fatty acid composition occurred gradually over the course of the experiment. Analyses were only done for samples up to four days after the start of the experiment. This gave clear trends; however, the time span the algae need to fully adapt to a given temperature and light intensity conditions could not be determined. It was still clearly observable that the temperature dependent adaption of FA profiles in chloroplasts is a slow

process. However, also rapid reactions could be observed in the changes of fatty acid profiles after six hours of cultivation. These converse reactions gave a decrease in polyunsaturation and an increase in saturation. For experiments with the same temperature they were more pronounced with increasing intensities. These converse reactions included an increase in the 16:0. 18:0 and 18:1 FAs, while all other FAs except for the ones present in low amounts decreased. Those three FAs are the starting point for all other long chain fatty acids and they are synthesised in the stroma of the chloroplasts (Heldt & Piechulla, 2010). Desaturases and elongases on the other hand are mostly anchored in the endoplasmic reticulum (Heldt & Piechulla, 2010). One explanation for the changes in the composition of fatty acids could be that either the *de novo* synthesis in the chloroplast stroma was enhanced as a reaction to increased light intensities, that the synthesised FAs could not be transported to the endoplasmic reticulum or that the desaturases anchored in the endoplasmic reticulum were inactivated. Another possibility would be that there is an active degradation of polyunsaturated fatty acids. In the case of the strong decrease of the 18:3 fatty acid, this could be explained by an active reduction of thylakoids. High light intensities pose a risk for plants, as they can generate reactive oxygen species which can destroy the cell (Heldt & Piechulla, 2010). After these initial converse reactions, slower adaptions of the fatty acid profiles took place, which seemed to be mainly dependent on cultivation temperature. The only experiment, where no converse reactions could be observed, was the one using high cultivation temperatures of 35 °C. However, this does not necessarily mean that those reactions were absent. It could also mean that they happened faster and at the six hour time point, slower temperature adaptions already took place. Higher temperatures are known to enhance plant metabolism and high intensities lead to efficient photosynthetic rates if temperatures are high as well (Berry & Bjorkman, 1980).

In the present experiments, the usage of red spectral light gave a higher degree of saturation than the other colours, making it relatively less useful to produce PUFAs. However, besides the synthesis of PUFAs, microalgae can also be used to produce high amounts of SFAs for the production of biodiesel (Hultberg et al., 2014b). Because of its high productivity, *A. obliquus* was reckoned to be a good candidate for biodiesel production (Abomohra et al., 2013; Breuer et al., 2012). This study showed that high cultivation temperatures gave high amounts of saturated fatty acids, although there seems to be a limit when it comes to increasing cultivation temperature. The fatty acid profile of *A. obliquus* grown at 35 °C did not have significantly more saturated fatty acids than for algae grown at 30 °C, while the high temperatures had negative effects on growth. Aiming at solving this problem, Grama et al. (2014) isolated a strain of *Acutodesmus* spp. in the Algerian Sahara, which they found to be adapted to high temperatures. It therefore had higher amounts of saturated fatty acids than strains adapted to colder temperatures. Applying different stressful conditions increased the total lipid content, but decreased growth. None of the stressors changed the fatty acid profile significantly but the conclusion was, that this strain of *Acutodesmus* spp. had the FA characteristics suitable for the production of high quality biofuel (Grama et al., 2014). In this study it was shown that the red light during cultivation gave high growth rates and in general higher saturation of fatty acids. Therefore, the amount of saturated fatty acid in the heat adapted strain of *Acutodesmus* spp. could possibly be increased by cultivating it under red light, which would make it even more suitable for the production of high quality biofuels.

7 Conclusion

Growth was mainly influenced by light intensities, with increasing photon flux density giving increasing biomass values. The results of this study also showed that either full solar spectrum light or green light was best for the cultivation of *Acutodesmus obliquus* at high intensities. Both higher and lower temperatures than the optimum temperature of *Acutodesmus obliquus* resulted in reduced biomass yields.

The fatty acid (FA) composition of *A. obliquus* was influenced by cultivation temperature, light intensities and spectral light. Light intensity seemed to induce strong but short lasting response in changes in the FA composition, which was strongest under red spectral white light. Differences between the microalgae's FA composition for blue, green and full spectral light were low, while red light gave a higher degree of saturation. Increased amounts of PUFAs and decreased amounts of SFAs were observed at moderate intensities and a low temperature (20°C), or at optimum temperature under high light intensities. For the commercial production of PUFAs, the results of this thesis lead to the recommendation of using high light intensities, a cultivation temperature of 30 °C and either full solar spectrum light or spectral green light. This combination appeared to promote high growth and a high production of PUFAs. The usage of green spectral light showed increased microalgae growth performance with increasing light intensities, therefore tests should be conducted using even higher light intensities to reveal if green light outperforms full solar spectrum light at intensities higher than those tested in the present experiments. The highest amount of PUFAs was achieved at a cultivation temperature of 20 °C, however growth in this experiment was low. To improve productivity, the microalgae should be cultivated under high light intensities and optimum temperatures until a high biomass is reached and should subsequentially be cooled down to 20 °C to increase the amount of PUFAs. The results of this thesis show that *Acutodesmus obliquus* has a high potential for commercial cultivation

to produce PUFAs for the food and feed industry. High intensities and a 30 °C cultivation temperature gave the best results in this context. Further studies should be conducted to reveal effects of optimized temperature profile in combination with spectral light to obtain efficient biomass production and targeted fatty acid production.

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10 Appendix

10.1 Example calculation for establishment of starter cultures

To start every experiment with the same conditions, the eleven test tubes are filled with a culture that should have an optical density of 0.2. The OD is measured of three replicates of the preculture. Then the inoculation volume to establish a four-litre starter culture can be calculated. This example shows the result for experiment E2, table 4 contains the example values for ODs:

Table 4: Example values for optical density, measured as triplicate.

	Rep 1	Rep 2	Rep 3	Average
OD	3.76	3.83	3.80	**3.80**

With the formula:

$$\frac{Required\ total\ volume\ \times Required\ OD}{OD\ of\ preculture}$$

$$\rightarrow\ \frac{4000\ ml\ \times 0.2}{3.8} = 211\ ml$$

This means 211 ml of preculture are needed on 4000 ml – 211 ml = 3.789 ml of growth medium.

10.2 Example calculation for optical density to dry weight correlation

For a concentrated algae solution and three dilutions, the values for optical density and dry weight were measured. Dividing the OD through the DW that was already divided through the used volume gives the correlation in [mg/ml]. Table 5 gives example values for the establishment of an OD-DW correlation.

Table 5: Example values for calculation of optical density to dry weight correlation.

Sample	Dilution	Avg OD	Avg DW [mg]	Used volume [ml]	OD-DW [ml/mg]	Avg OD-DW
A	None	6.47	3.33	1	1.94	2.05
B	1:2	3.24	5.07	3	1.92	
C	1:4	1.57	3.87	5	2.03	
D	1:8	0.69	3.00	10	2.32	

For sample C the calculation would be:

$$\frac{1.57}{3.87\ mg/5\ ml} = 2.03\ ml/mg$$

The average of all OD-DW correlations is used for further calculation of dry weights with the use of optical density.

10.3 Example calculation for required volume for lipid extraction

With the OD-DW correlation, the required volume of a sample with a known OD for the lipid extraction can be calculated. For each sample, a dry weight of 2.5 mg was analysed. The following formula was used:

$$\frac{2.5\ mg\ \times OD - DW\ \frac{ml}{mg}}{OD}$$

For experiment E2 the OD-DW was 2.07 ml mg^{-1}, for a sample with an OD of 1.724, this gave a required volume of:

$$\frac{2.5\ mg\ \times 2.07\ \frac{ml}{mg}}{1.724} = 3.0\ ml$$

10.4 Fatty acid profile graphs

10.4.1 Experiment E1 (low intensities, 30 °C)

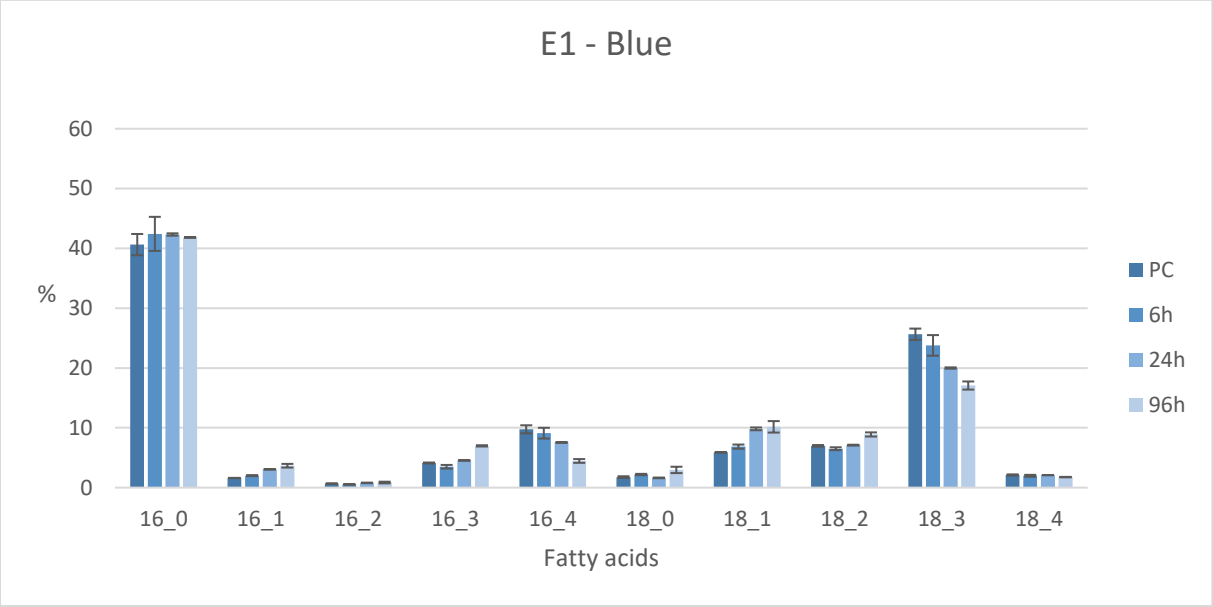


Figure 42: Development of the fatty acid composition of the blue light group in experiment E1 (low intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

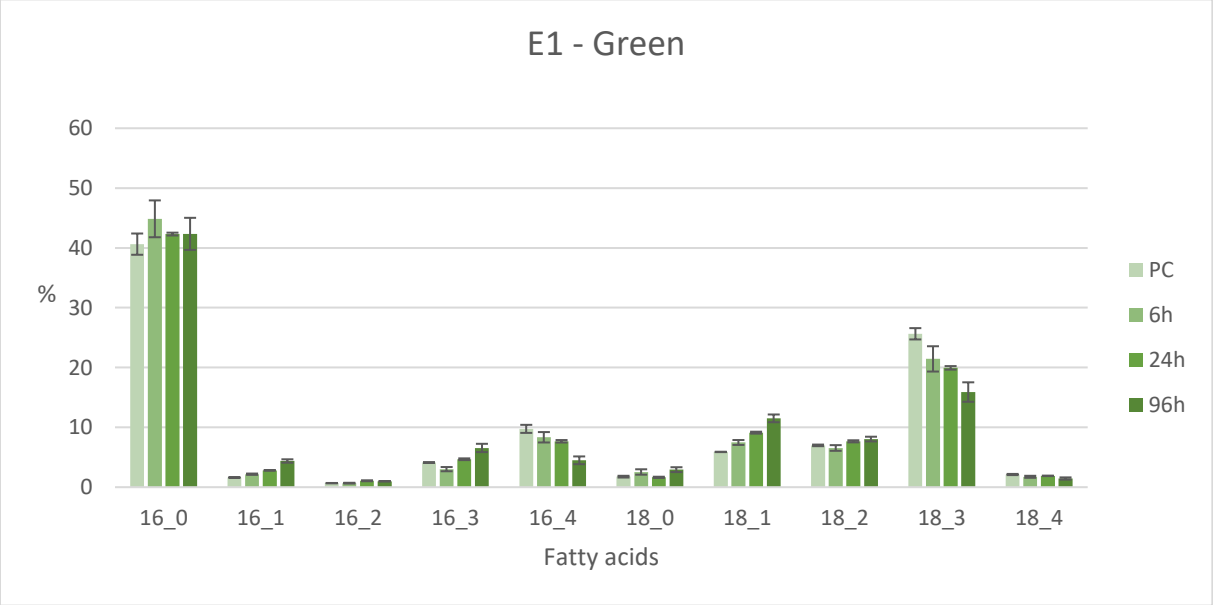


Figure 43: Development of the fatty acid composition of the green light group in experiment E1 (low intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

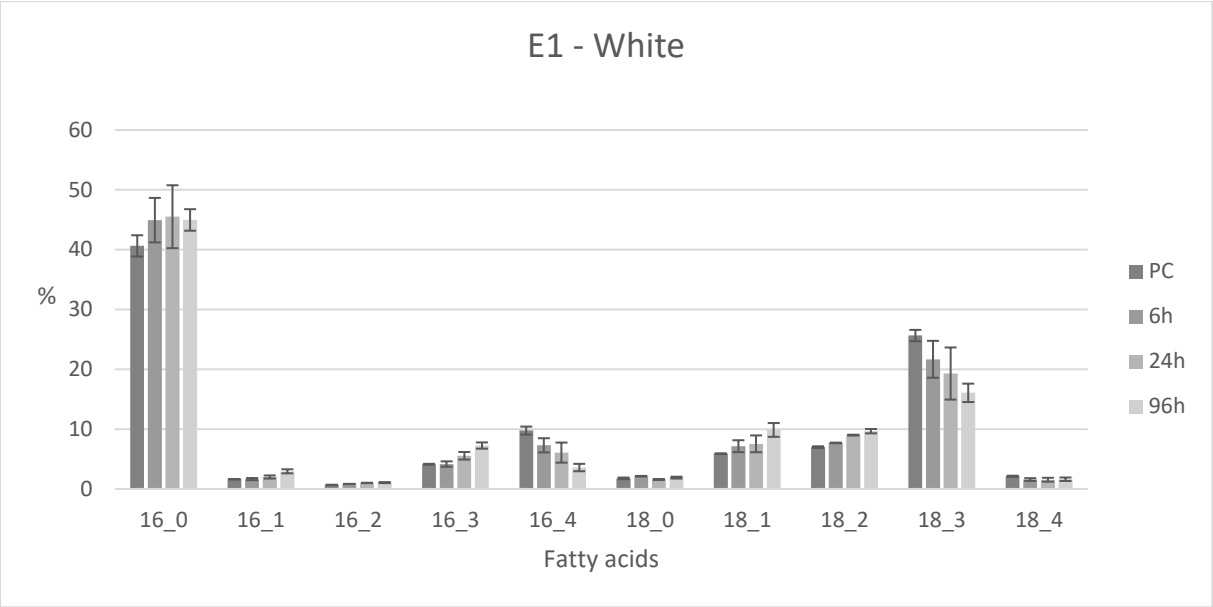


Figure 44: Development of the fatty acid composition of the white light control group in experiment E1 (low intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

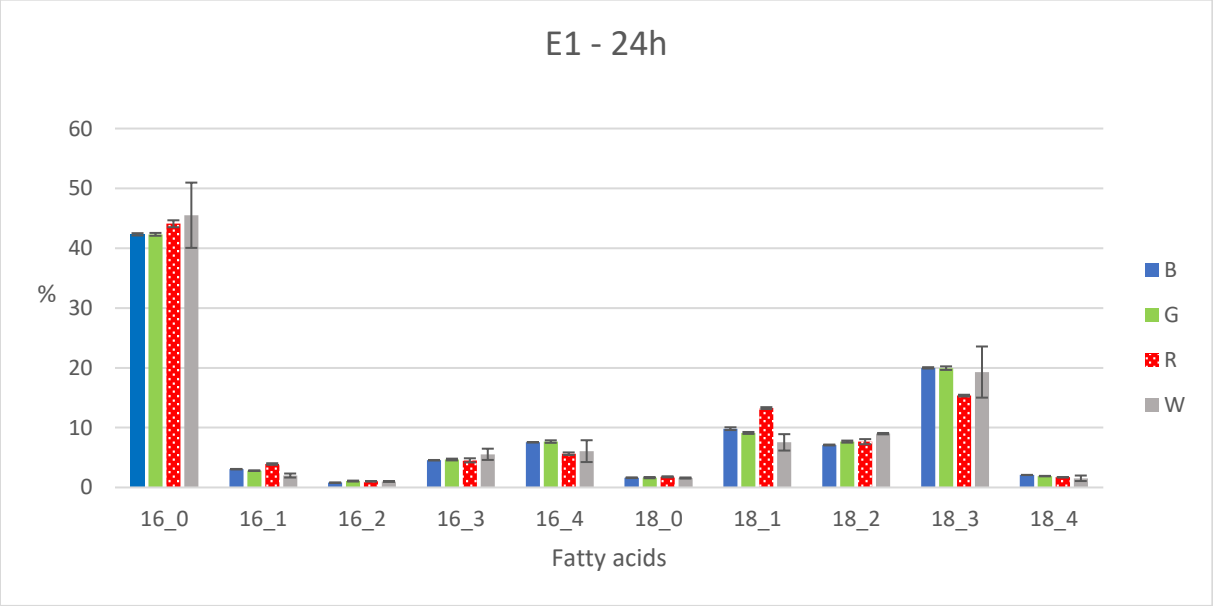


Figure 45: Comparison of the fatty acid composition of experiment E1 (low intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 24 hours after start of cultivation, including standard deviation bars.

10.4.2 Experiment E2 (moderate intensities, 30 °C)

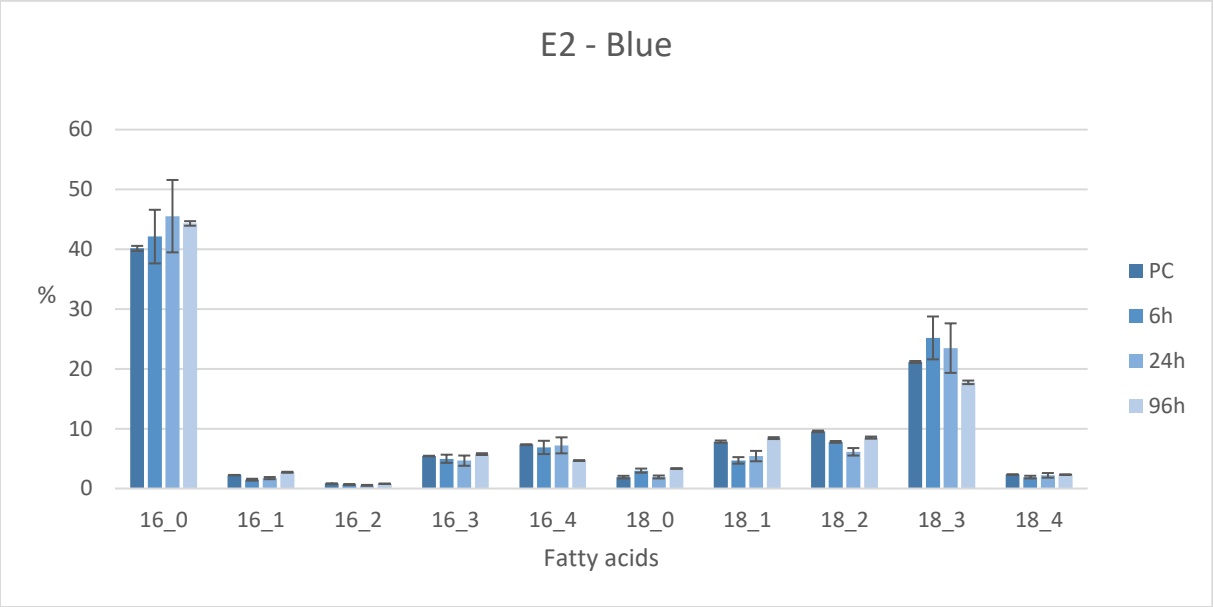


Figure 46: Development of the fatty acid composition of the blue light group in experiment E2 (moderate intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

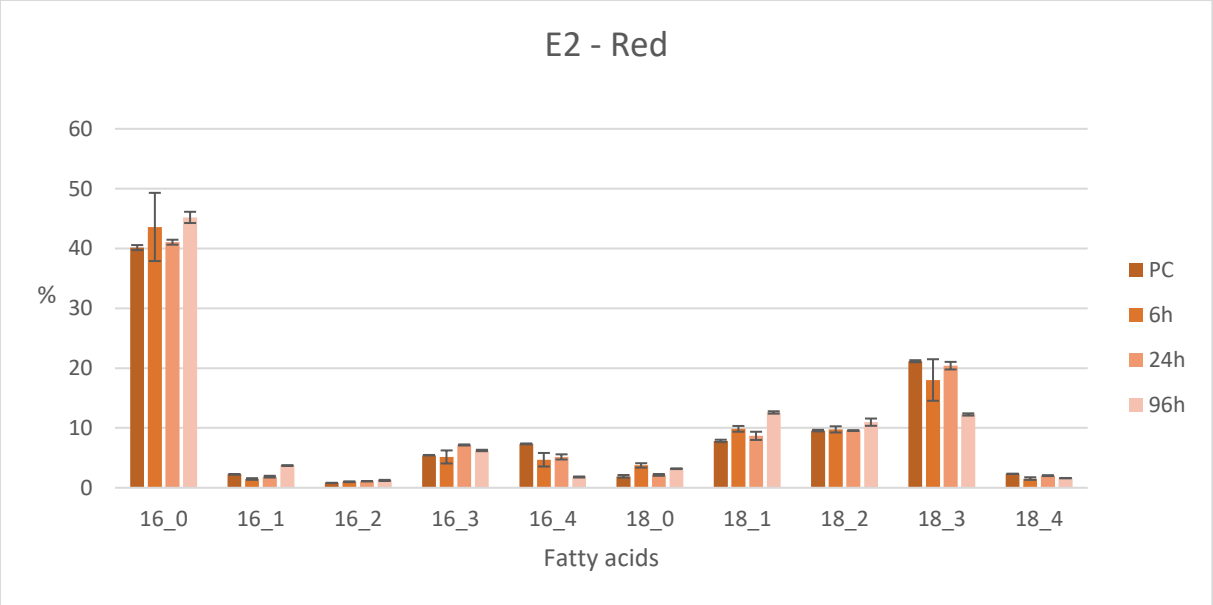


Figure 47: Development of the fatty acid composition of the red light group in experiment E2 (moderate intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

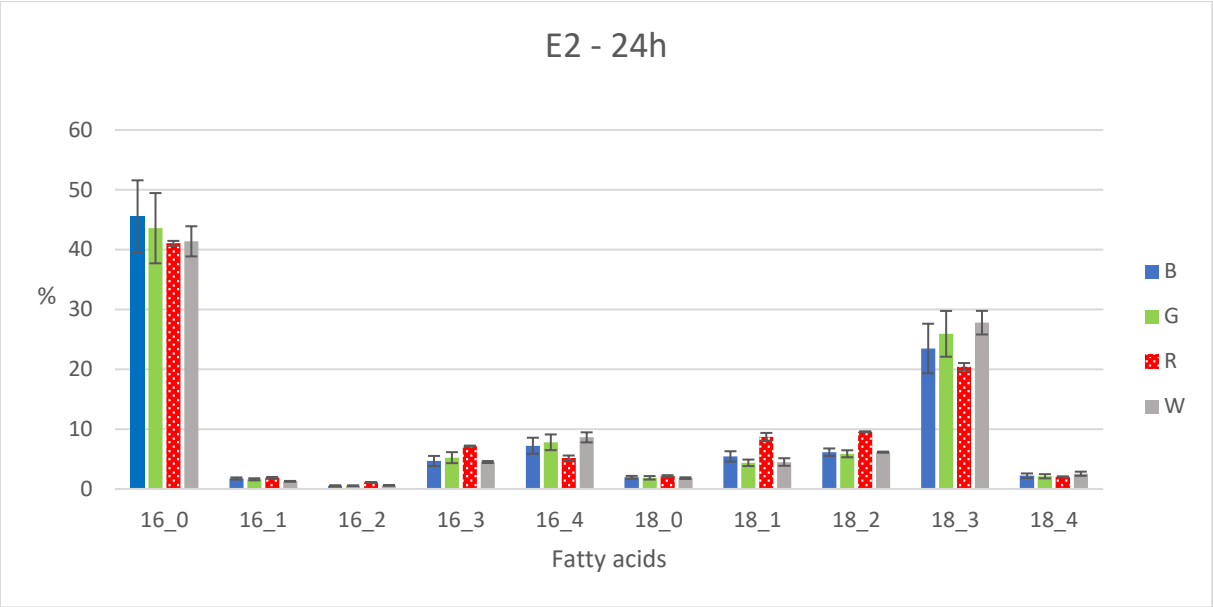


Figure 48: Comparison of the fatty acid composition of experiment E2 (moderate intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 24 hours after start of cultivation, including standard deviation bars.

10.4.3 Experiment E3 (moderate intensities, 20 °C)

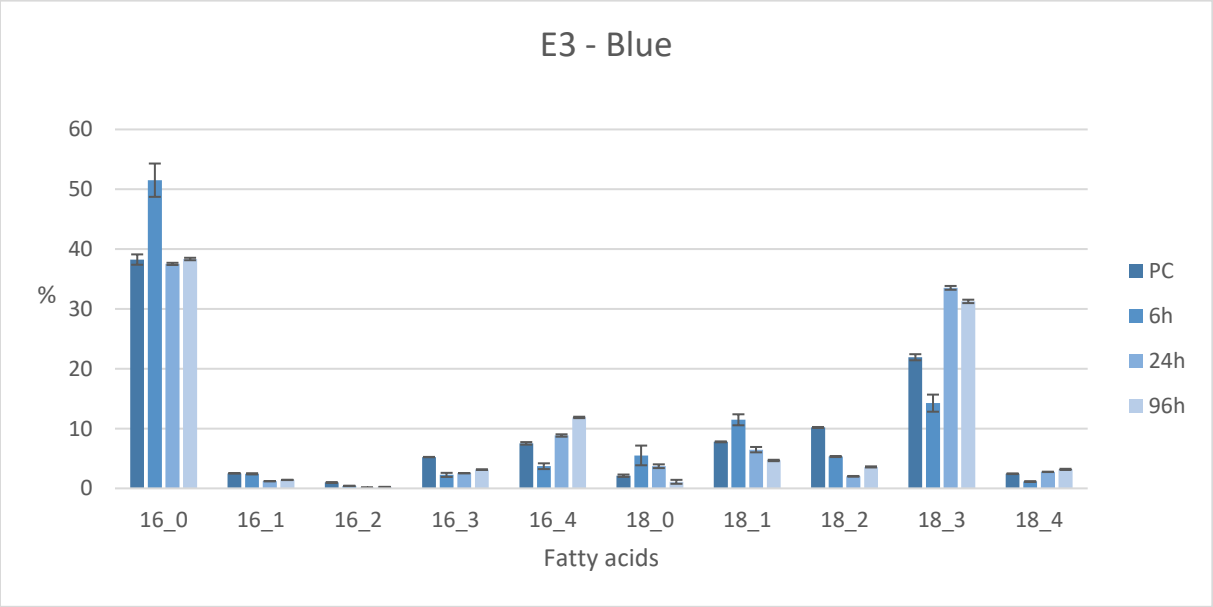


Figure 49: Development of the fatty acid composition of the blue light group in experiment E3 (moderate intensities at 20 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars

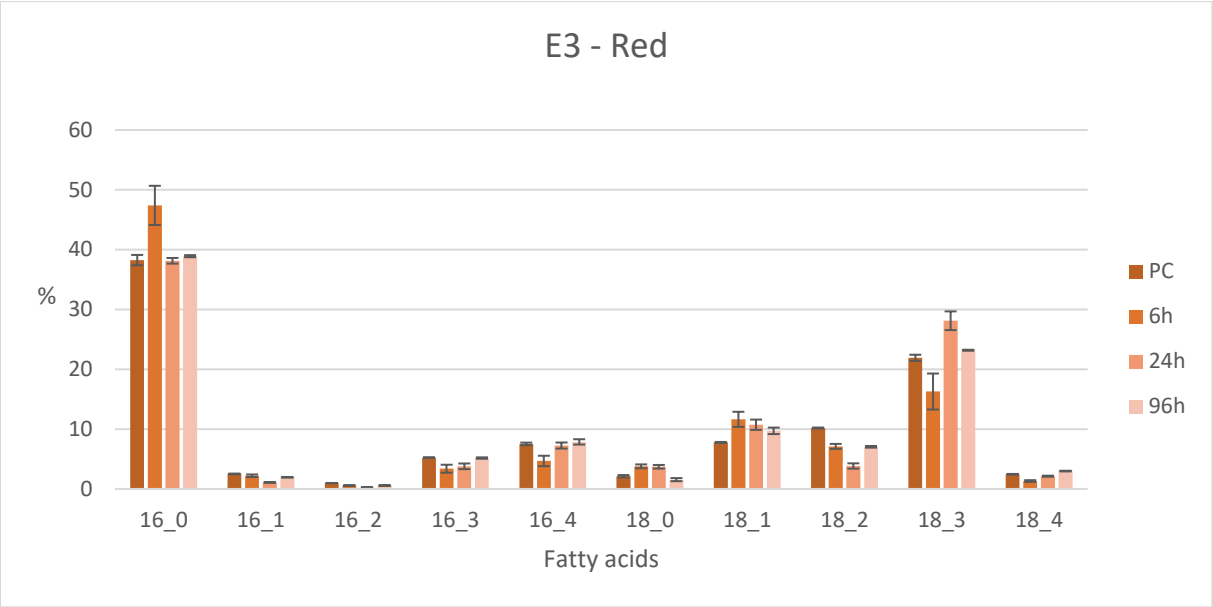


Figure 50: Development of the fatty acid composition of the red light group in experiment E3 (moderate intensities at 20 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars

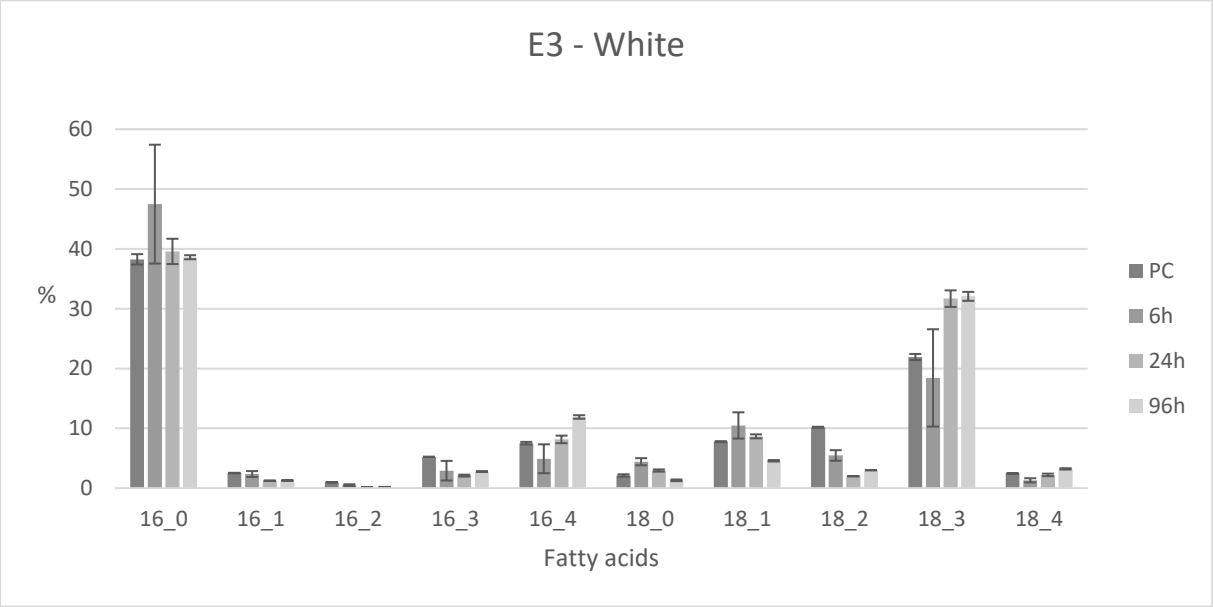


Figure 51: Development of the fatty acid composition of the white light control group in experiment E3 (moderate intensities at 20 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars

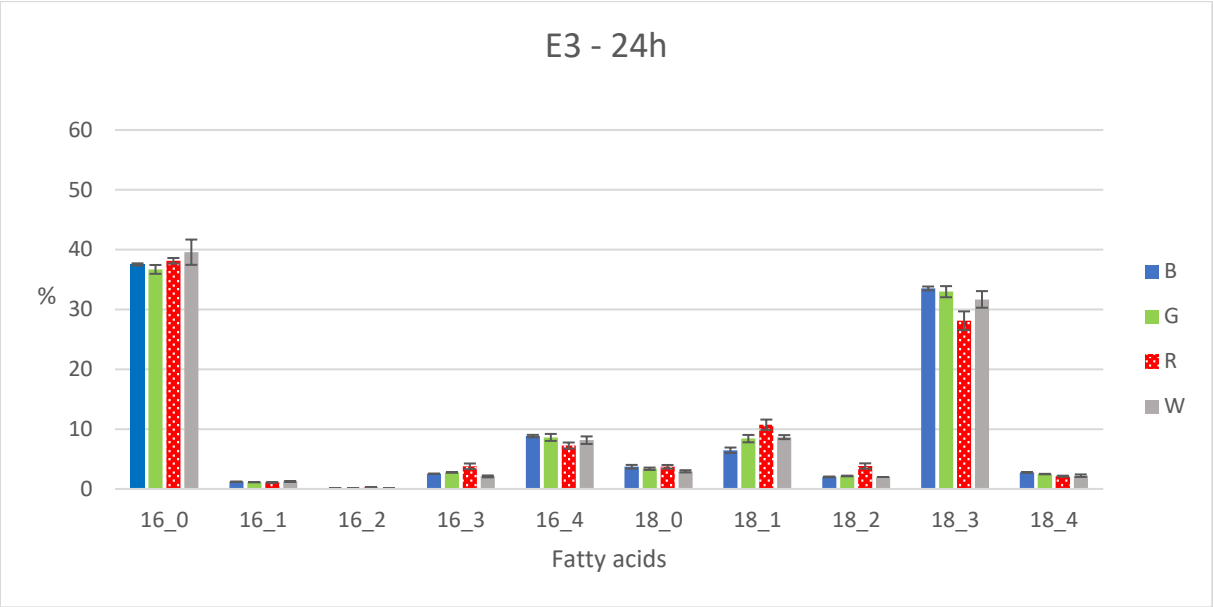


Figure 52: Comparison of the fatty acid composition of experiment E3 (moderate intensities, 20 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 24 hours after start of cultivation, including standard deviation bars.

10.4.4 Experiment E4 (moderate intensities, 35 °C)

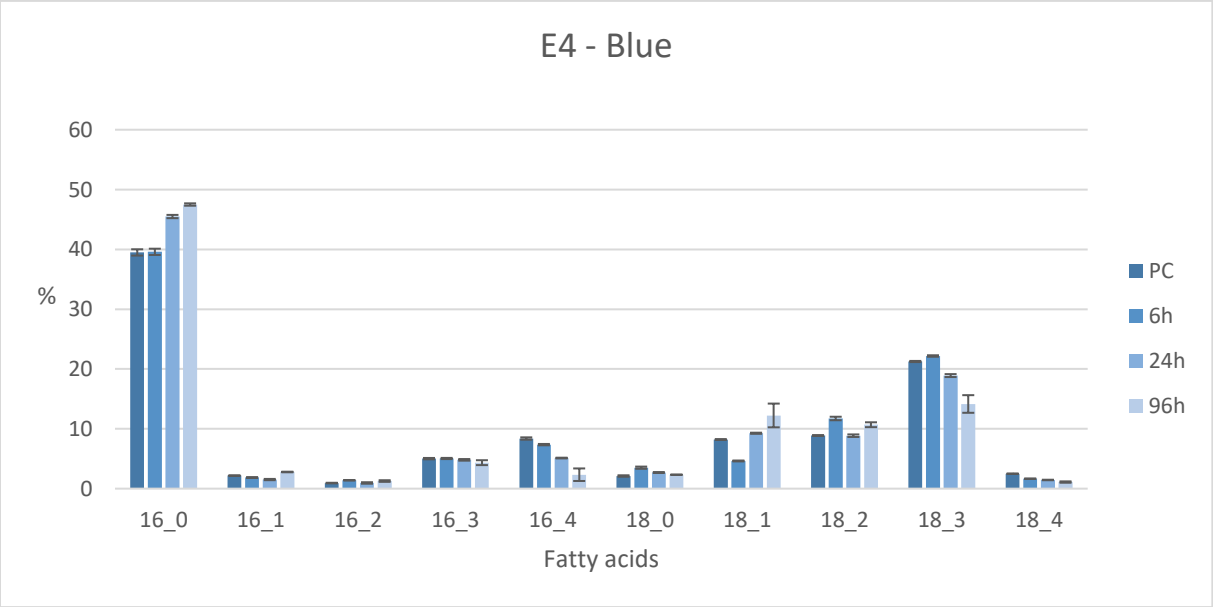


Figure 53: Development of the fatty acid composition of the blue light group in experiment E4 (moderate intensities at 35 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

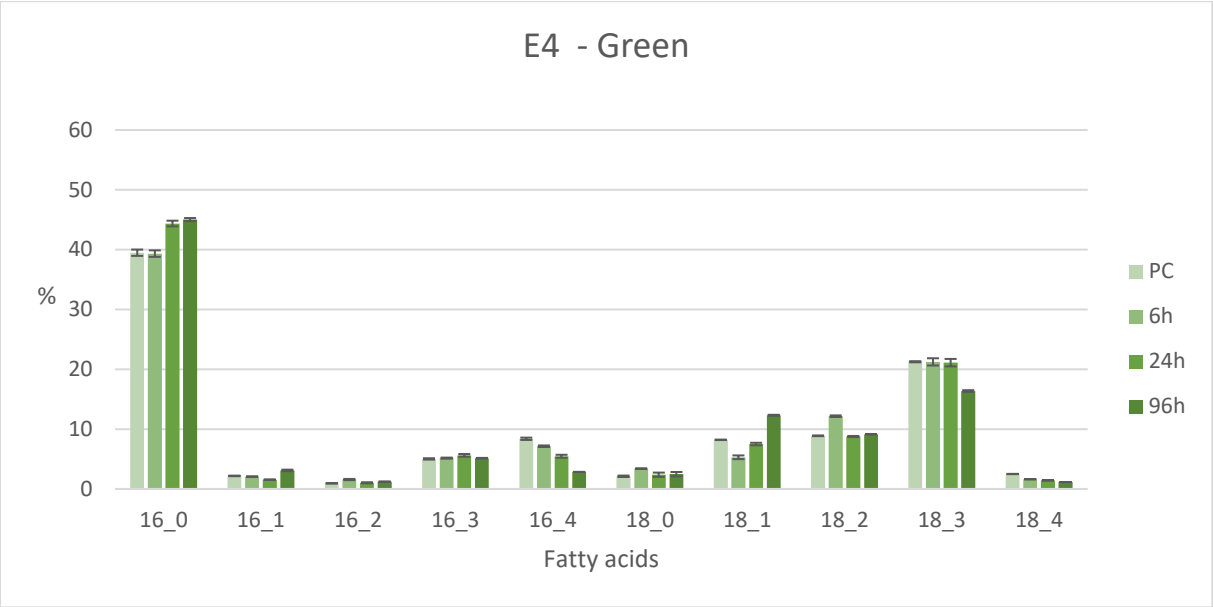


Figure 54: Development of the fatty acid composition of the blue light group in experiment E4 (moderate intensities at 35 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

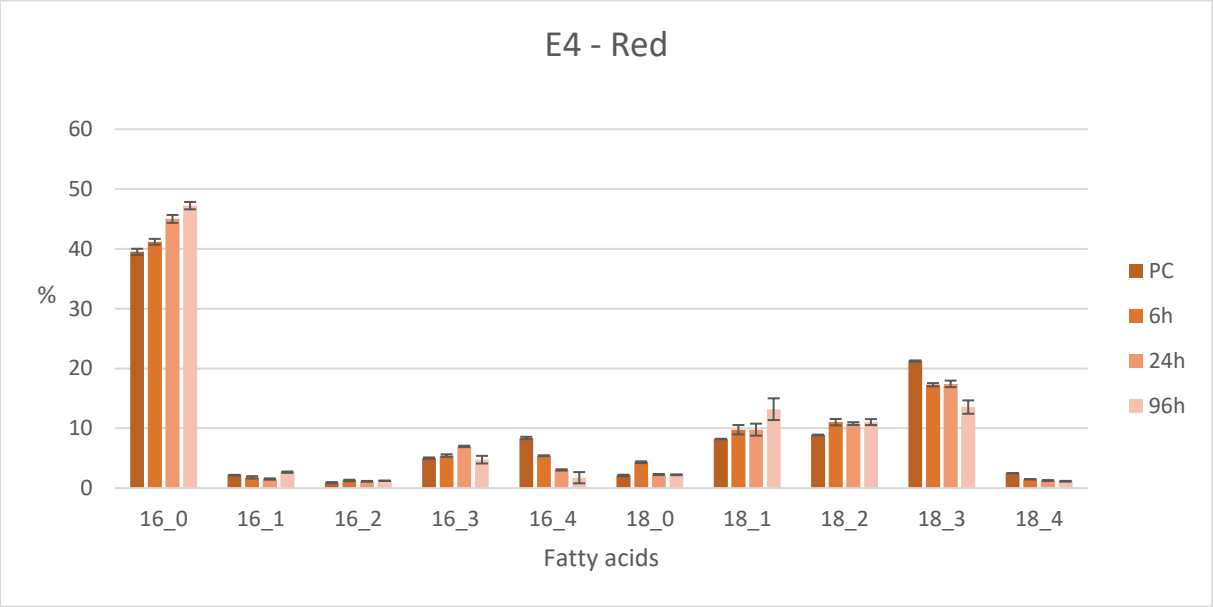


Figure 55: Development of the fatty acid composition of the blue light group in experiment E4 (moderate intensities at 35 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

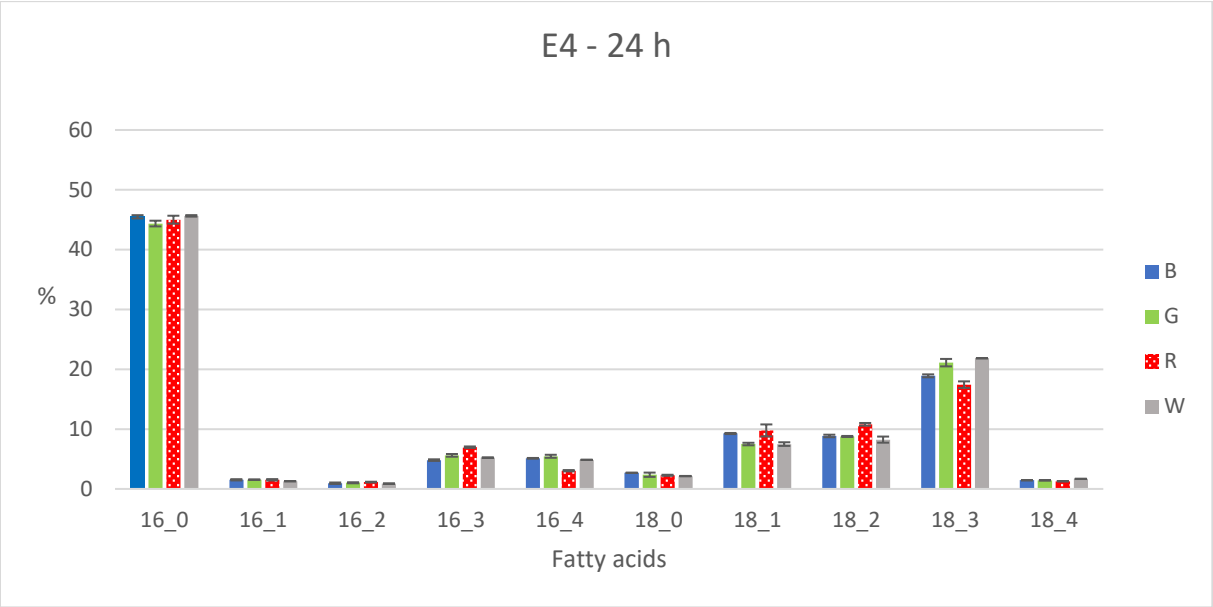


Figure 56: Comparison of the fatty acid composition of experiment E4 (moderate intensities, 35 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 24 hours after start of cultivation, including standard deviation bars.

10.4.5 Experiment E5 (high intensities, 30 °C)

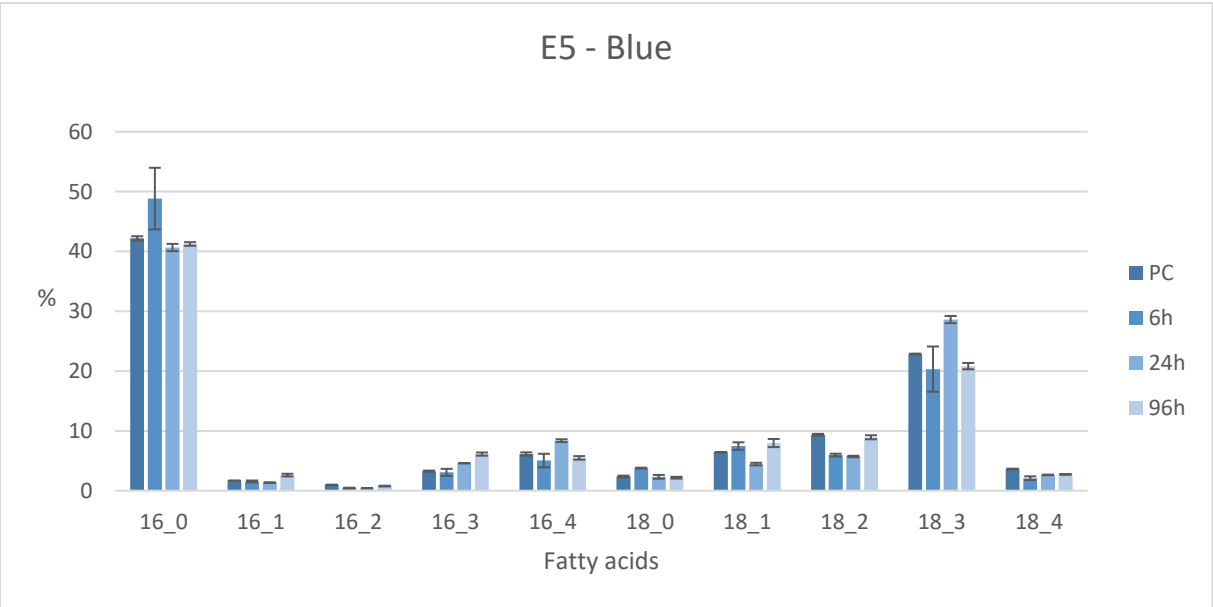


Figure 57: Development of the fatty acid composition of the blue light group in experiment E5 (high intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

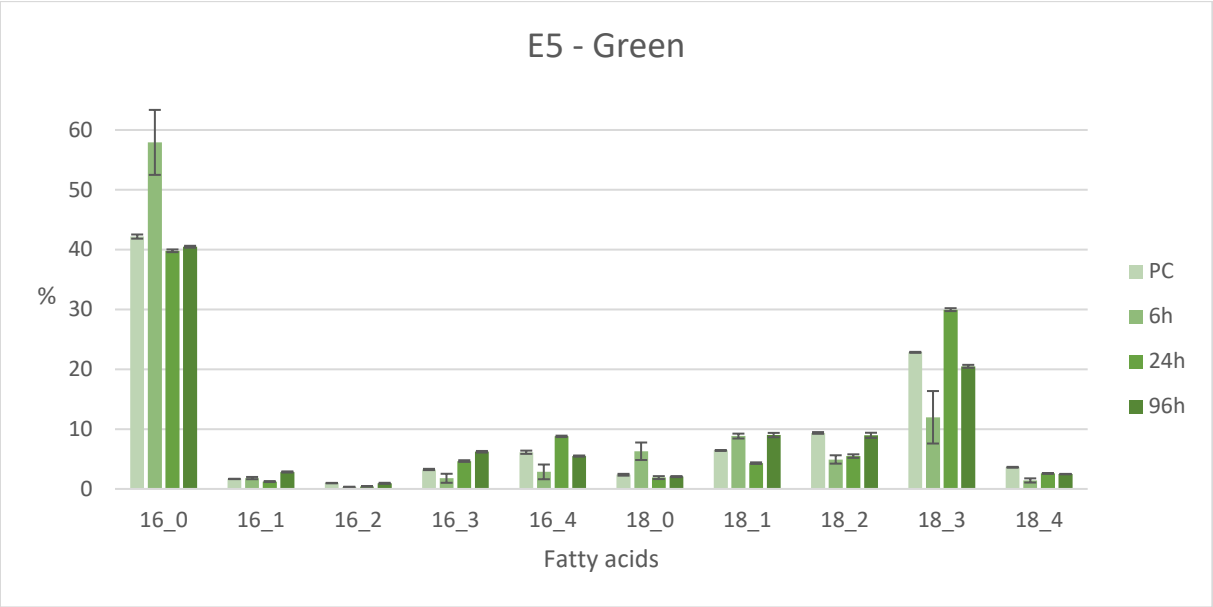


Figure 58: Development of the fatty acid composition of the red light group in experiment E5 (high intensities at 30 °C) over time (6, 24 and 96 hours) compared to the preculture, including standard deviation bars.

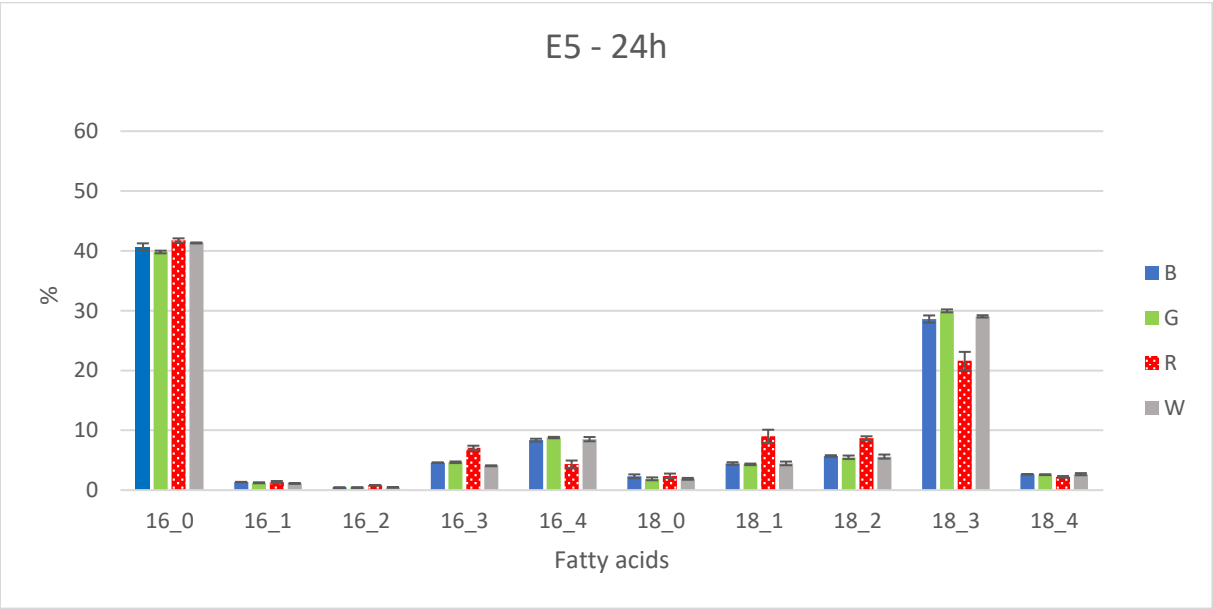


Figure 59: Comparison of the fatty acid composition of experiment E5 (high intensities, 30 °C) between the blue (B), green (G) and red (R) colour group and the white light control group (W), at the time point of 24 hours after start of cultivation, including standard deviation bars.

Table 6: Comparison of the average amount of percentages for each fatty acid for the colour groups blue (B), green (G) and red (R) and the white light control group for the experiments E1 to E5. Each fatty acid is colour coded, with the darkest shade of green indicating the maximum value of the fatty acid and light to no colour indicating minimum values.

Fatty acid	Preculture	E1				E2				E3				E4				E5			
		B	G	R	W	B	G	R	W	B	G	R	W	B	G	R	W	B	G	R	W
16_0	40,14	41,82	42,34	43,04	44,96	44,32	42,49	45,19	52,10	38,34	37,69	38,91	38,60	47,49	45,03	47,22	46,76	41,24	40,49	42,65	41,66
16_1	2,04	3,66	4,38	4,58	2,94	2,72	2,71	3,71	3,26	1,42	1,31	1,94	1,27	2,78	3,10	2,67	1,74	2,61	2,83	3,72	2,68
16_2	0,87	0,85	0,99	1,03	1,05	0,80	0,89	1,22	1,00	0,24	0,21	0,57	0,19	1,27	1,21	1,23	1,17	0,77	0,93	1,22	0,98
16_3	4,61	6,98	6,56	6,81	7,25	5,76	6,25	6,23	3,81	3,13	3,30	5,16	2,77	4,34	5,14	4,76	4,50	6,12	6,21	6,90	5,36
16_4	7,82	4,46	4,49	2,33	3,57	4,68	5,07	1,80	3,30	11,86	12,08	7,86	11,89	2,33	2,84	1,74	2,69	5,48	5,49	1,85	5,05
18_0	2,05	2,96	2,93	2,02	1,89	3,34	3,80	3,18	3,73	1,10	1,21	1,55	1,31	2,33	2,46	2,23	2,25	2,17	2,05	2,06	2,21
18_1	7,23	10,16	11,50	15,33	9,86	8,43	8,05	12,59	9,58	4,66	4,33	9,71	4,57	12,23	12,31	13,20	10,90	7,97	9,00	13,64	8,72
18_2	8,99	8,89	8,04	9,53	9,65	8,51	8,09	10,96	7,90	3,59	3,08	7,04	3,00	10,68	9,14	11,04	9,91	8,93	8,96	12,02	10,33
18_3	22,56	17,07	15,90	12,77	16,06	17,75	19,17	12,25	12,10	31,26	32,57	23,18	32,06	14,15	16,36	13,55	16,91	20,81	20,50	13,27	19,36
18_4	2,60	1,76	1,45	1,35	1,62	2,32	2,11	1,60	1,66	3,17	2,99	2,98	3,22	1,10	1,15	1,14	1,49	2,72	2,48	1,63	2,58

10.5 Statistics

10.5.1 Significance table Y(II)

Table 7: Significance values of t-tests between the red (R), blue (B) and green (G) colour group for Y(II) values of the experiments E2 to E5. Fields are colour coded with p-values smaller than 0.05 having a yellow field colour.

t-test	E2	E3	E4	E5
R vs. G	0,2814	0,5255	0,5286	0,3351
R vs. B	0,0978	0,7179	0,0507	0,0848
B vs. G	0,198	0,1381	0,094	0,0049

10.5.2 Significance tables growth

Table 8: Significance values of t-tests between the red (R), blue (B) and green (G) colour group for biomass values at different time values of the experiments E1 to E5. Fields are colour coded with p-values smaller than 0.05 having a yellow field colour. Fields are empty where no significances could be calculated due to missing triplicates.

E1	time [h]	R vs G	R vs B	G vs B
t-test	0	ns	ns	ns
	3	ns	ns	ns
	6	0,003	0,035	0,225
	24	0,009	0,017	0,032
	48	0,010	0,024	0,245
	72	0,002	0,003	0,284
	96	0,021	0,019	0,076
	123	0,014	0,028	0,563
	166	0,091	0,086	0,860

E2	time [h]	R vs G	R vs B	G vs B
t-test	0	ns	ns	ns
	1	0,3414	0,1399	0,706
	3	0,0176	0,0288	0,0669
	6	0,0078	0,0131	0,0848
	24	0,0152	0,0306	0,1317
	48	0,0107	0,0077	0,0108
	72	0,0451	0,0512	0,2536
	96	0,2211	0,0235	0,0118
	118,5	0,0752	0,0066	0,0142
	164	0,3202	ns	ns

E3	time [h]	R vs G	R vs B	G vs B
t-test	0	ns	ns	ns
	3	ns	ns	ns
	6	0,0512	0,0267	0,8845
	24	0,4647	0,2079	0,0351
	48	0,0073	0,0399	0,013
	72	0,1367	0,0198	0,0417
	96	0,1192	0,0216	0,0313
	166	0,1464	0,0067	0,0722

E4	time [h]	R vs G	R vs B	G vs B
t-test	0	ns	ns	ns
	1	0,5799	0,874	0,0572
	3	0,4817	0,7342	0,2854
	6	0,209	0,2487	0,7952
	24	0,1237	0,0307	0,2748
	48	0,0282	0,0001	0,0232
	72	0,1405	0,0509	0,1273
	96	0,0567	0,01	0,0009

E5	time [h]	R vs G	R vs B	G vs B
t-test	0	ns		
	1	0,1853		
	3	0,2667		
	6	0,332		
	24	0,3782		
	48	0,6685		
	72	0,9832		
	96	0,0669		

Table 9: Significance values of t-tests between the red (R), blue (B) and green (G) colour group for biomass values at 96 hours between the experiments E1, E2 and E5 (same cultivation temperature, different light intensities). Fields are colour coded with p-values smaller than 0.05 having a yellow field colour. Fields are empty where no significances could be calculated due to missing triplicates.

	Red	Green	Blue
E1 vs E2	0,0066	0,0006	0,0003
E1 vs E5	0,0039	0,0033	
E2 vs E5	0,0575	0,0094	

Table 10: Significance values of t-tests between the red (R), blue (B) and green (G) colour group for biomass values at 96 hours between the experiments E2, E3 and E4 (same light intensity, different cultivation temperature). Fields are colour coded with p-values smaller than 0.05 having a yellow field colour. Fields are empty where no significances could be calculated due to missing triplicates.

	Red	Green	Blue
E2 vs E3	0,0362	0,0051	0,0006
E2 vs E4	0,0438	0,0151	0,0135
E3 vs E4	0,0731	0,0483	0,055

10.5.3 Significance table fatty acids

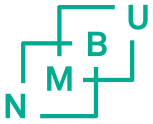
Table 11; Significance values of t-tests between the red (R), blue (B) and green (G) colour group for the most important fatty acids (average amounts of more than 5 %) of the experiments E1 to E5. Fields are colour coded with p-values smaller than 0.05 having a yellow field colour. Fields are empty where no significances could be calculated due to missing triplicates.

E1	t-test	16_0	16_3	16_4	18_1	18_2	18_3
	B vs G	0,7666	0,4233	0,962	0,178	0,0013	0,1357
	B vs R	0,0131	0,3421	0,0106	0,0197	0,0344	0,0204
	G vs R	0,6664	0,469	0,0252	0,0069	0,0085	0,2982
E2	t-test	16_0	16_3	16_4	18_1	18_2	18_3
	B vs G	0,0391	0,0672	0,0152	0,1688	0,1128	0,6819
	B vs R	0,3186	0,0838	0,0002	0,0004	0,0211	0,0025
	G vs R	0,0436	0,8678	0,0002	0,0023	0,0236	0,1908
E3	t-test	16_0	16_3	16_4	18_1	18_2	18_3
	B vs G	0,0208	0,0046	0,0006	0,0139	0,0128	0,4706
	B vs R						
	G vs R						
E4	t-test	16_0	16_3	16_4	18_1	18_2	18_3
	B vs G	0,0092	0,0789	0,4899	0,9564	0,0212	0,1171
	B vs R	0,4221	0,5621	0,6586	0,7029	0,5602	0,726
	G vs R	0,0319	0,419	0,1771	0,4734	0,0233	0,4762
E5	t-test	16_0	16_3	16_4	18_1	18_2	18_3
	B vs G	0,0726	0,7132	0,9719	0,0293	0,8867	0,3231
	B vs R	0,0021	0,0119	0,0007	0,0094	0,0097	0,001
	G vs R	0,0098	0,1078	0,0023	0,0101	0,0088	0,1539

11 Statutory declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted, either literally or by content from the used sources.

date: _____ signature: _____



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