

Green Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

UV-C-mediated lipid induction and settling, a step change towards economical microalgal biodiesel production

Kalpesh Sharma, Yan Li and Peer M Schenk*

Algae Biotechnology Laboratory, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

*Corresponding author email: p.schenk@uq.edu.au

Abstract

Microalgae are highly efficient primary producers that can be grown in most types of water on non-arable land as a promising source of biofuel. However, large-scale microalgal biofuel production is currently uneconomical due to slow growth of high-percentage oleaginous algae and large harvesting costs. Here we present a new strategy, using a small dose of externally applied UV-C radiation, that significantly increases lipid contents of fast growing microalgae and that at higher doses also results in rapid settling. The procedure essentially separates biomass growth from lipid accumulation and harvesting which was tested in several microalgal strains and optimized to be completed within 48 h for *Tetraselmis* sp using pilot-scale outdoor cultivation. This process resulted in a significant increase of both volumetric and areal lipid productivity with higher polyunsaturated fatty acids contents, while considerably reducing harvesting costs. Other benefits include control of co-cultured microbes and sanitized water for recirculation. UV-C-mediated lipid induction and settling (LIS) may contribute to commercial microalgal biofuel production.

Broader context

Biofuel production by microalgae is considered a promising approach but that is currently uneconomical due to slow-growing high-lipid producing microalgal strains and high harvesting costs. The first problem is essentially that microalgae cannot grow rapidly and produce large amounts of lipids simultaneously. The second problem is that microalgae are microscopic organisms that are currently mostly harvested by centrifugation, a very expensive and energy-intensive step that is not easily scalable. Here we present a new approach that addresses both problems. UV-C radiation was used as an easily-applied external

stimulant to rapidly induce lipids and also as a new method to induce overnight settling of microalgae for harvesting (a step that would normally require expensive flocculants or centrifuges).

Introduction

With growing tension on arable land and irrigation water resources, the case for algae as future food source, CO₂ sequestration medium, and biofuel supply becomes more compelling¹⁻⁴. New technologies need to produce an additional 5-6 billion tons of organic carbon apart from agricultural crops⁵. Large-scale microalgae cultivation is considered one of the most promising feedstocks to produce biofuels without competing for food production or biodiverse natural landscapes; recent progress and future perspectives have been intensively reviewed³⁻⁷. Microalgae can also be cultivated in brackish or seawater and as an integrated concept with wastewater treatment to optimize the energetic, nutrient and financial input for feedstock production⁸. However, high capital costs, low lipid productivity of fast-growing microalgae and high harvesting & processing costs are major bottlenecks, hindering commercial production of microalgal oil-derived biodiesel. To obtain high-lipid contents in fast-growing microalgae, typically, lipid induction techniques need to be applied, but these may slow down growth and add extra costs⁹. Harvesting is another critical and challenging stage in mass microalgae cultivation due to low biomass concentrations (typically 0.3-0.5 g dry weight (DW) L⁻¹)^{10, 11}, large volumes of water, and small cell sizes¹²⁻¹⁶. Harvesting consumes 20-30% of biomass production costs^{13, 17, 18}, is alga-specific and may comprise different physical, chemical and biological processes^{11, 12, 18, 19}.

Synthesis and accumulation of large amounts of triacylglycerides (TAG) in microalgae is required for biodiesel production by transesterification. Cellular lipid and TAG induction techniques (either acting individually or in combination include nutrient stress, osmotic stress, radiation, pH and temperature change, heavy metals and other chemicals, and some metabolic engineering approaches^{9, 20-26}. Nitrate starvation is most widely-studied in almost all candidate biofuel microalgae⁹ and is easily-applied by omitting nitrate in the growth medium or letting the culture use up nutrients. However, it typically takes 3-5 days until significant amounts of lipids are synthesized which is accompanied by slow growth rates and thus finally affects total biomass and lipid productivity²⁷. Change in temperature, pH, salinity and heavy metals are difficult to regulate at large-scale. Genetically-modified microalgae potentially produce more lipids, but regulatory issues increase costs, while microalgae naturally high in lipids or hydrocarbons are typically slow-growing²⁸.

Thus there is demand for a process that can not only rapidly induce lipids in exponentially growing algae, but also bridge the gap between the time taken in microalgal biomass production to harvesting the biomass. After considering different lipid induction techniques, we concluded that, external lipid induction with light irradiation is a promising approach that does not involve any alternation in growth media, or leave traces in the biomass and that, moreover, can be easily dosed. Solar ultraviolet light comprises UV-A (400-315 nm; 3.10–3.94 eV/photon), UV-B (315-280 nm; 3.94–4.43 eV/photon), and UV-C (280-100 nm; 4.43–12.4 eV/photon), but the latter does not reach the Earth's surface. UV-A and UV-B have already been tested for microalgae, but lipid induction took more than 2-3 days to show an effect^{22, 24, 29-31}. As UV-C light carries more energy per photon, the hypotheses was to use UV-C (253 nm) radiation as a stress induction technique after reaching a certain cell density, to minimize biomass loss and obtain high-lipid productivity. Several microalgae were tested and the oleaginous marine microalga *Tetraselmis* sp. M8 has been chosen as the main strain for this study, based on its previous identification as a candidate strain for biofuel feedstock, including comparatively rapid dominant growth under mid-scale outdoor conditions (growth rate $\mu=0.47$) and a polyunsaturated fatty acids (PUFA) profile suitable for biodiesel production³². Our results demonstrate that UV-C stress not only led to doubling of cellular lipid contents but also the loss of flagella and subsequent settling, a convenient, time-saving and cost-effective way for microalgae harvesting.

Methods

Laboratory-scale microalgae culturing and UV-C treatment

Strain *Tetraselmis* sp. M8 was collected in an intertidal rock pool at Maroochydore, Australia (26°39'39"S 153°6,18"E; 12 pm on 6 August 2009³²). Pure cultures of isolate M8 were grown in f/2 medium⁴⁸ in autoclaved artificial sea water (35 ppt NaCl). *Tetraselmis chui*, *Chlorella* sp. BR, *Nannochloropsis* sp. BR2, and *Dunaliella salina* strains have been previously described³². Laboratory culturing conditions were set at 23°C on an orbital shaker (Thermoline) at 100 rpm with 12 hours of light and dark rhythm. When the cell density reached 10⁶/mL, the culture was used for UV-C radiation trials. Primary stock cultures were maintained aerobically in 100 mL Erlenmeyer flasks with constant orbital shaking (100 rpm) at 25°C, under a 12:12 h light/dark photoperiod of fluorescent white light (120 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). Nitrate and phosphate levels were determined using the corresponding API Nutrient

testing kit according to the manufacturer's instructions. After gently stirring, 5 mL aliquots of M8 culture were pipetted into a Petri dish, forming a thin layer inside (total of 20 plates). Plates were randomly divided into five groups with three plates used for each for UV-C radiation (253 nm) treatment in a UV chamber (BioRAD, Gs-Genelinker). They were separately irradiated at 0, 100, 250, 500 and 1000 mJ/cm². All Petri dish cultures were subsequently incubated for 24 h. Then the algae survival rate was measured by counting the live cells based on visibly intact chloroplasts in each replicate. The cell size was also measured by compound microscopy (Olympus).

Lipid fluorescence analysis

For each replicate, 1 mL of algae cells was sampled and stained with 3 μ L Nile Red solution (a 10 mg/mL Nile Red (Sigma, USA) stock solution was prepared in acetone and stored in the dark at 4°C). After 20 min incubation in the dark, the lipid fluorescence intensity of cells was detected by fluorescence-activated cell analyzing (BD LSR II: Analyzing flow cytometer) with 573 nm of excitation wavelength. A total of 10,000 cells were counted in each sample. A gate was set up to separate the fluorescence-activated cells and inactivated cells based on the analysis of cells without Nile Red staining. The background absorbance from chloroplast auto-fluorescence was subtracted from each sample using mock-treated (control) *Tetraselmis* sp. M8. Qualitative observation was conducted simultaneously by fluorescence microscopy as described below. For plate reader analyses samples were stained with Nile red stock solution as mention above and 200 μ L of each sample was loaded into 96 well-plates and analyzed with a Fluostar optima and Polarstar optima (BMG LAB tech) plate reader at excitation and emission wavelength of 485/584 nm; the gain was set at 2400.

GC-MS analyses

GC-MS analysis was carried out as described previously³². Briefly, 4 mL of algal culture was centrifuged at 16,000xg for 3 min. The supernatant was discarded and the lipid in the algal pellet was hydrolyzed and methyl-esterified with 300 μ L of 2% H₂SO₄ methanol solution at 80°C by shaking (480 rpm) for 2 h on a thermalmixturer (Eppendorff). Prior to esterification, 50 μ g of heneicosanoic acid (C21) was added to the pellet in each sample as an internal standard (IS). After esterification, 300 μ L of 0.9% (w/v) NaCl and 300 μ L of hexane (analytical grade) were added and vortexed for 20 s. Phase separation was

performed by centrifugation at 16,000xg for 3 min and the hexane layer was used for lipid profile analysis. GC/MS analyses were carried out on an Agilent 6890 GC coupled to a 5975 MSD. A DB-Wax column (Agilent, 122-7032) was used with running conditions as described in Agilent's RTL DBWax method (Application note: 5988-5871EN). Identification of fatty acid methyl esters was based on mass spectral profiles and retention times in the Agilent's RTL DBWax method. Each fatty acid methyl ester (FAME) was quantified using the formula:

$$\text{Fatty acid } (\mu\text{g/mL}) = (\text{integral of fatty acid} / \text{integral internal standard}) \times (\text{molecular mass of fatty acid} / \text{molecular mass of internal standard}) \times 50/4$$

Enzymatic assays

Lipid peroxidation, superoxide dismutase (SOD) and glutathione reductase (GR) activities were determined using the TBARS, Superoxide Dismutase or Glutathione Reductase (GR) Assay Kits, respectively, (Cayman) according to the manufacturer's instructions. A total of 5 mL culture with 10^6 cells/mL was used as UV-C treated cells (0–1000 mJ/cm²) and a mock-treated control culture were centrifuged at 2000xg and resuspended in 1 mL diluted assay buffer provided by the manufacturer. The cultures were then sonicated three times for 5 min at 40 V setting on ice, centrifuged at 1500xg for five min at 4°C and the supernatant was used to perform the assay.

UV-C-mediated microalgal lipid biosynthesis induction and settling (LIS)

A total of 20 L of exponentially-grown culture of *Tetraselmis* sp. M8 were taken from a 1000 L raceway pond and poured in two 50 L-aquarium fish tanks, each containing 10 L of culture (Supplementary Fig. 5). One of the tanks was UV-C-radiated for 15 min (3 J/cm²) with a custom-built UV-C chamber with continuous bubbling to ensure that all cells were evenly radiated, while the other tank was a mock-treated control culture without UV-C radiation that was bubbled for the same time. A total of 5 mL of the culture from both the tanks was taken into 20 mL Falcon tubes for Nile red-staining and cell counting. The culture was left overnight for settling and lipid induction. Cell count was carried out at 3 h and 15 h after treatment.

Outdoor raceway cultivation

In order to evaluate the UV-C lipid induction and settling technique of microalgae in a mid-scale outdoor setting, 20 L of laboratory-grown *Tetraselmis* sp. M8 culture was used to inoculate two identical 1000 L outdoor raceway ponds built by The University of Queensland's Algae Biotechnology Laboratory (www.algaebiotech.org; Supplementary Fig. 7). Mid-scale outdoor raceway experiments were conducted between January and September 2012, with most experiments carried out during winter time under sunny conditions when average day temperatures ranged from 22°C–26.5°C. Cultures were continuously grown in 1000 L-outdoor raceway ponds and climatically adapted before experiments commenced. Cultures were continuously grown in seawater containing f/2 medium at uncontrolled pH values of 8.8-9.1 and cell culture densities of $1.5\text{--}2.3 \times 10^6/\text{mL}$. Cell counts were conducted daily followed by Nile red fluorescence and GC-MS analyses during controlled experiments. Cultures were also checked daily under the microscope to ensure that no contamination with other microalgae occurred.

After initial optimization experiments in the outdoor raceway ponds, 500 L of microalgae culture with 12 cm water depth was used for 6 h (72 J/cm^2) of UV-C treatment. This dosage was found suitable to induce lipid biosynthesis with minimum cell death. Subsequent experiments were set up for 8 days out of which typically by day 3 and 4 the culture was under N starvation and after which an increase in fluorescence of Nile red-stained cells could be measured. To further minimize initial cell mortality, the UV-C dosage was divided into two phases. An initial dose of UV-C was applied on day 5 for 4 h followed by 36 h continued cultivation for rapid lipid induction. A final dose of UV-C was applied for 2 h at the end of day 7, culture-mixing was stopped and the culture was harvested on the next morning after overnight settling. The experiment was repeated three times and included a raceway swap, but included a reduced frequency of measurements.

Dry weight measurement

At the end of outdoor cultivation, just before harvesting, 25 mL of the culture was used for dry weight measurements. The 25 mL culture was filtered through a $0.27 \mu\text{m}$ glass fiber filter (Millipore) which was pre-weighed and pre-washed with 1 mL distilled water in a vacuumed filter unit (three biological replicates were used from each culture (UV-C treated and mock-treated control)). After filtration the

filters were kept in individual Petri dishes to avoid contamination and dried in a drying oven for 24 h at 80°C with the plate lid half open. After 24 h the filters were immediately weighted.

To determine the dry weight the following formula was used:

$$\text{Dry weight mg/L} = \frac{\text{Filter dry weight} - \text{Filter pre-weight}}{\text{Filtered volume mL} \times 1000}$$

Dry weight in mg/L was determined from the average of three weight measurements for each replicate.

Microscopic analyses

After a lipid induction phase, microalgae cells were stained with 2 µg/mL Nile red (dissolved in acetone; Sigma, USA) for 15 min and photographed using a fluorescent Olympus BX61 microscope fitted with a 100 W High Pressure Mercury Burner and an Olympus DP10 digital camera. Differential interference contrast (DIC) and epifluorescent (excitation: 510–550 nm, emission: 590 nm) images were obtained at 1000× magnification with oil immersion.

UV-C disinfectant effect

On the morning on day eight of the experiment, just before harvesting, the top layer of both the raceways was collected and 100 µL of the culture was spread on LB plates and PDB plates to screen for bacteria and fungal colonies present after the experiment. The bacterial plates were incubated in a 37°C growth chamber for 48 h, whereas fungal plates were incubated for 7 days at room temperature. Microbial growth was monitored every 24 h.

Analytical methods

Measurement of nitrate and phosphate levels in the photobioreactor was performed using colorimetric assays (API, Aquarium Pharmaceuticals and Nutrafin, respectively). Data for growth rates and lipid productivity was statistically analyzed by one-way analysis of variance (ANOVA) with different microalgal cultures as the source of variance and growth rate or lipid productivity as dependent variables. This was followed by Turkey's multiple comparisons test ($P > 0.05$) where appropriate. Student's t-test was used for pairwise comparisons.

Construction of UV-C chamber

For scaling up of the UV-C lipid induction and settling technique, a UV-C chamber was custom-built using a stainless steel hood of a plant growth chamber light lamp in which the original fluorescent tubes were replaced with six G8T5 (TUV 8W) GE Philips Bi-Pin 288, UV-C (253 nm) lamps connected in series (Supplementary Fig. 5A). The height, length and width of the chamber was 40 cm, 45 cm and 40 cm, respectively. Based on a comparison to the specifications of the laboratory UV chamber (BioRAD, Gs-Genelinker), the heat losses of the lamps and the set-up of the outdoor chamber, 12.5% of the energy was estimated to reach the culture surface as UV-C radiation (approx. $12 \text{ J/cm}^2 \text{ h}^{-1}$; actual UV-C surface radiation was not measured).

Techno-economic analysis

Using the time taken to harvest 1,000 L of *Tetraselmis* sp. M8 culture in mid-scale outdoor cultivation and information from previously carried out studies and specifications provided by companies that supply the equipment and chemicals, a theoretical calculation was carried out to determine the techno-economic feasibility of overall biomass recovery in a one-step as well as a two-steps method. For the costing purpose, harvesting of 10,000 L of *Tetraselmis* culture was considered (Table 1). Assessments were independently developed in accordance with Australian conditions and where possible, were compared to equivalent costing from previous economic analyses of microalgae biofuel systems ¹¹.

Results

UV-C-irradiation induces microalgal lipid biosynthesis

Microalgae have been suggested as a promising source of triacylglycerides for biodiesel production. *Tetraselmis* sp. M8 microalgal cells had previously been shown to accumulate significant amounts of lipids after nutrient deprivation ³². Preliminary experiments using UV-C radiation on various microalgal cells, including *Tetraselmis* sp. M8, indicated that this external stress can also stimulate lipid accumulation (data not shown). To test whether both, nutrient starvation and UV-C treatment can lead

to further lipid biosynthesis, combined sequential stress treatments were carried out. Nile red-staining of nitrogen-deprived *Tetraselmis* sp. M8 cells indicated that an additional external stress treatment by UV-C exposure, led to an increase in cell sizes and additional lipid accumulation in lipid bodies within 24 h (Fig. 1). As UV-C doses were increased from 100 mJ/cm² to 250 mJ/cm², fluorescence intensities became stronger, but at higher doses cell rupturing was observed. Interestingly, detached flagella were found at UV-C radiation doses at and above 100 mJ/cm² (arrow in Fig. 1a) and this could also occasionally be observed under a fluorescence microscope with blue light excitation within a few seconds. As expected, a sharp decline in cell survival rates was observed with increased UV-C radiation (Fig. 1b). The cell survival rate was approximately half (LD50) at 250 mJ/cm² (Fig. 2).

To profile and quantify lipid accumulation of millions of individual nutrient-starved *Tetraselmis* cells simultaneously following different UV-C exposure, a flow cytometer was used (Fig. 2). With an increase of UV-C radiation, algal cells were divided into two different populations, termed P1 and P2, according to a background fluorescence intensity cutoff in the unstained control population. The majority of the P1 population in this control can be attributed to auto-fluorescence of chloroplasts (Supplementary Fig. 1a). In the untreated, but Nile red-stained control, 60% of the cells were allocated to the P1 population, whereas 40% were in the P2 population (Fig. 2a; Supplementary Fig. 1b); the latter can be attributed to lipid accumulation by nutrient deprivation in these cells. When UV-C radiation of 100 or 250 mJ/cm² was used for treatment of nutrient-starved cells, P1 was reduced to 40%, while the lipid-accumulating P2 increased to 60%, indicating a marked increase in lipid production after UV-C treatment (Fig. 2a; Supplementary Fig. 1c,d). However, at 500 mJ/cm² P1 and P2 were equal and at 1000 mJ/cm² P1 further increased to 65% while the high lipid-containing P2 decreased to 35% (Fig. 2A; Supplementary Fig. 1e,f). This is consistent with microscopic observations, suggesting that high UV-C doses damaged cells to a point where most cells were unable to produce Nile red fluorescence-detectable lipids. Quantification based on average fluorescence intensities from three separately-grown and -treated microalgal populations, showed that P1 cells always had much less fluorescence intensities than the lipid-accumulating P2 populations (Fig. 2b). Overall, fluorescence intensities of both populations combined, confirmed that nutrient-starved UV-C-treated cells (100 mJ/cm²) had significantly increased lipid contents ($P=0.022$; Fig. 2c). However, there was no significant fluorescence increase between the untreated control and cells treated with 250 mJ/cm² or higher doses which can probably be attributed to cell damage leading to cell rupturing and release of lipid bodies that were not quantified by flow cytometry.

Fatty acid profiles of UV-C-treated microalgae shift towards unsaturated fatty acids

To further quantify the ability of UV-C stress to increase cellular TAG contents and to profile the fatty acid composition, GC-MS analyses were performed. These confirmed the results obtained from flow cytometry, showing a significant total fatty acid increase ($P=0.032$, $P=0.014$; respectively) of UV-C-treated cultures (100 and 250 mJ/cm^2) compared to untreated controls, whereas cultures treated with 500 and 1000 mJ/cm^2 showed no significant difference (Fig. 2d). Moreover, in cultures treated with 100 and 250 mJ/cm^2 , the amount of unsaturated fatty acids (USFA) significantly increased and also the proportion of USFA compared to total fatty acids (Fig. 2d). Amongst the saturated fatty acids (SFA), the highest increase was observed for C16 (palmitic acid), followed by C14 (Supplementary Fig. 2). On the other hand, C18 and C20 did not show any increase in cultures treated with 100 and 250 mJ/cm^2 UV-C, whereas in cultures treated with 500 and 1000 mJ/cm^2 the amount of C20 was significantly higher ($P=0.0239$, $P=0.0164$, respectively). When comparing different USFA, cultures treated with 100 and 250 mJ/cm^2 UV-C showed significant increases for all detected USFA, most notably C16:4, C18:1 cis+trans, C18:3n3 and C20:5 (Supplementary Fig. 3).

It appears plausible that UV-C stress leads to cellular lipid biosynthesis to improve survival of microalgal cells, as this has been observed for many other stresses, most of which lead to a higher proportion of SFAs⁹. To better understand the underlying mechanisms for the observed shift of fatty acids towards unsaturated fatty acids following UV-C treatment, several enzymatic assays were carried out. Decomposition of unstable peroxides derived from PUFAs resulting in the formation of malondialdehyde (MDA), was quantified colorimetrically following its controlled reaction with thiobarbituric acids (TBARS). Supplementary Figure 4a shows that as the UV-C radiation increased from 0-1000 mJ/cm^2 the formation of MDA increased in samples and was highest at 1000 mJ/cm^2 . Similarly, superoxide dismutase (SOD) and glutathione reductase (GR) activities increased with higher UV-C doses (Supplementary Fig. 4b,c), suggesting that UV-C-treated cells underwent oxidative stress causing damage to DNA and cellular membranes. Hence the increased amount and proportion of USFA in UV-C-stressed cells may be required for repair of cellular damage to membranes and to provide sufficient antioxidant capacity to restore redox homeostasis.

UV-C treatment leads to settling of flagellate algal cultures

While conducting UV-C lipid induction experiments with cultures of the flagellate microalgae *Tetraselmis* sp. M8, it was observed that cells exposed to UV-C radiation also detached their flagella and showed other signs of cell damage (Fig. 1). The effect could also be observed under blue light using fluorescence microscopy and for other flagellate microalgae (data not shown). When applied to entire laboratory-grown cultures, this resulted in settling of the algal cells if left undisturbed. Hence the notion of combining lipid induction with settling for microalgae harvesting was developed. The process of exposing flagellate algal cultures to UV-C irradiation is therefore from here-on referred to as Lipid Induction and Settling (LIS). To test whether this is applicable to outdoor conditions, algal culture settling experiments were performed on saturated 20 L-cultures grown in open-lid aquariums using a specially-designed UV-C chamber (Supplementary Fig. 5). Within 3 h of LIS treatment, about 90% cells settled when compared to untreated cultures and within 15 h >99% cells settled (Supplementary Fig. 6).

Upscaling of lipid induction and settling (LIS)

Following initial optimization of UV-C-mediated LIS to determine a suitable dose of UV-C, experiments were conducted on two pilot-scale 1000 L-raceways ponds (Supplementary Fig. 7). Both ponds were built with the exact same specifications for optimal comparisons. Cultures were first pre-adapted to growth in these ponds for 2 months where they were maintained in late exponential growth phase. For the experiment, the same culture was split into two identically-built raceways and grown simultaneously where one of the raceways was UV-C-treated and the other was mock-treated. The trials commenced at a cell density of 1.5×10^6 /mL (day 1; Fig. 3). By day 3 cells had reached the nutrient depletion point (nitrate and phosphate not measurable) and days 4 and 5 were intended for nutrient stress induction. During the early morning on day 5, an initial dose of UV-C was applied for 4 h (48 J/cm^2). As a result, the cell count dropped slightly from 1.7×10^6 to 1.5×10^6 in the UV-C-treated raceway (Fig. 3a). After 36 h of lipid induction phase, a final dose of UV-C was applied in the evening of day 7 for 2 h (24 J/cm^2) to induce loss of flagella and settling and Cells were left for overnight sedimentation. In the morning, more than 93% of UV-C-treated cells had settled and the remaining cell count of the culture dropped to 10^5 /mL, whereas the mock-treated raceway contained 1.5×10^6 cells/mL (Fig. 3b). Along with cell density, lipid fluorescence from Nile red-stained cells and fatty acid profiles were determined at different stages of the experiment (Fig. 4). From day 5, a clear increase in lipid fluorescence was observed, reaching a

maximum on day 6 that was twice as high in the UV-C treated raceway (Fig. 4a). As the cell density in UV-C-treated cultures was slightly lower than in the control, values for total lipids per cell were significantly higher in UV-C-treated culture on day 6 compared to the control (Fig. 4b).

As expected, the total DW of harvested biomass obtained from UV-C-treated raceways was marginally reduced compared to the control (Fig. 5a). However, the total fatty acid yield, determined by GC-MS was significantly higher (300 $\mu\text{g}/\text{mL}$) when compared to the control (200 $\mu\text{g}/\text{mL}$; Fig. 5b). Whereas, total lipid productivity was also significantly ($P < 0.05$) higher (35 $\text{mg}/\text{L}/\text{day}$) when compared to the control (23 $\text{mg}/\text{L}/\text{day}$) (Supplementary Fig. 8). Consistent with the lab-scale studies, the highest increase in fatty acids could be attributed to USFA contents ($P = 0.028$), whereas the total SFA increase was not significant ($P = 0.058$; Fig. 5c,d). Specifically, consistent with the lab-scale studies, the highest increases were observed for C16, C16:3, C16:4, C18:4 (Supplementary Fig. 2 and 3).

UV-C treatment aids in water sanitization for reculturing

To investigate whether UV-C treatment had an effect to bacterial and fungal growth in cultures, both groups of organisms were quantified in UV-C-treated vs. untreated used growth medium from outdoor raceway ponds. As expected, the UV-C-treated raceway pond harbored significantly-reduced culturable bacteria and fungi (Supplementary Fig. 9).

Techno-economic analysis shows reduced costs for primary dewatering

A techno-economic analysis was carried out to determine whether the implementation of LIS would result in significant cost savings compared to other currently-used methods for algal biomass harvesting. As shown in Table 1, an estimated 89% and 45% of the costs for primary dewatering of *Tetraselmis* sp. M8 can be saved for flocculant-assisted settling or dispersed-air-flotation, respectively, when using LIS instead. When considering lipid productivity rather than algal biomass, LIS stood further out as a cost-effective method as this would further reduce costs on a per lipid basis. Alternatively, cost savings maybe achieved by shortened lipid induction periods of nutrient- and UV-C-stressed cultures, compared to cultures stressed by nutrient depletion alone. Another advantage of LIS compared to flocculant-assisted methods, is that no chemical residues remain in the harvested biomass. Full life cycle analyses

should be conducted on optimized large-scale cultivation systems to determine how actual costs for algal biomass and lipid production compare to other feedstocks.

Discussion

This study first introduced UV-C radiation to stimulate lipid biosynthesis in microalgae. It highlights the efficiency of UV-C radiation on lipid induction and also provides a benchmark for UV-C-induced settling of flagellate microalgae. Maximum lipid induction was depicted by cultures radiated at 100 mJ/cm² and 250 mJ/cm² on Petri dishes (Fig. c,d), and 48 J/cm² was found suitable for 12 cm-deep *Tetraselmis* outdoor raceway cultures containing 1.5x10⁶ cells/mL (Fig. 4 and 5). UV-C-induced settling occurred overnight. It should be considered that UV-C may also cause an increase of mutations in the culture. To avoid adaptation to repeated UV-C exposure all the culture was harvested after the experiment.

Coincident with studies conducted with UV-B radiation^{33, 34}, an increase of cell size was found (Fig. 1a; Supplementary Fig. 1e,f). A study on nitrogen deprivation in *Dunaliella tertiolecta* also noted increased cell size following lipid accumulation³⁵. So it was understood that the cell size increased by UV-C as a result of lipid induction. However, larger cells may display higher UV stress tolerance as suggested by UV-B research^{33, 34}. Cellular lipids may simply provide more energy reserves for stress responses. Alternatively, a UV-induced morphological variation maybe better adapted to adverse condition/lipid production. Since UV causes genetic mutations^{36, 37}, UV may have acted as a selection pressure during algal evolution^{38, 39}. At high UV-C doses, the release of lipid bodies became apparent as cell structures disintegrated (Fig. 1). Considering the difficulties often experienced for lipid extraction due to rigid cellular structures, UV-C-implemented cell damage may also contribute to higher lipid extraction efficiencies.

Compared to previous lipid induction techniques^{9, 40, 41}, UV-C radiation resulted in faster lipid biosynthesis stimulation within 24 h (Fig. 2-4). Depending on the quantification method used, the total lipid or TAG production approximately doubled compared to lipid induction by nutrient depletion only. Under outdoor conditions, TAGs were induced by lesser extend (Fig. 5b), perhaps because controls also received UV-containing solar irradiation or because biomass was harvested after the peak in lipid fluorescence of Nile red-stained cells (Fig. 4a). This emphasizes that further fine-tuning of UV-C exposure

and harvesting times may be required to maximize TAG induction while minimizing cell mortality, and this is likely to vary for different microalgae, cultivation systems, and climatic conditions.

Although the total lipid content increased by UV-C radiation to different extents, an alteration of fatty acid profiles only occurred at low doses (100 and 250 mJ/cm² for laboratory- and 48 J/cm² for outdoor-grown cultures). Interestingly, the decrease of C18 and C20 SFAs corresponded to the increase of C16:2, C16:4, C18:1, C18:2, C18:3 and C20:4 USFAs (Fig. 5; Supplementary Fig. 2, 3 and 9). A similar result was obtained in UV-B-treated *Spirulina*, concomitant with deleterious effects on thylakoid membrane integrity and protein profiles⁴². Amongst USFAs, the increment of PUFAs was the main change in the present study. As PUFAs are involved in cell repair and growth⁴³, it is conceivable that *Tetraselmis* cells attempted to repair the photo-damage caused by UV-C. However, photo-damage appeared irreversible at high UV-C doses since the lipid profile did not change in 24 h after 500 or 1000 mJ/cm² exposure.

The fatty acid synthesis pathway of *Tetraselmis* sp. is regarded to be similar to that of *Chlamydomonas reinhardtii*⁴⁴ where fatty acid desaturation results in insertion of double bonds into pre-formed fatty acid chains. Therefore, we speculate that the increase of C16:2, C16:4, C18:1, C18:2, C18:3 and C20:5 unsaturated fatty acids were actually the products of the desaturation of C16, C18 and C20 SFAs in *Tetraselmis* sp. M8 (Supplementary Fig. 10). Low UV-C dosage may help to convert SFAs to unsaturated fatty acids with a significant increase in PUFAs. In microalgae, the deleterious effect of UV light on thylakoid membrane integrity leads to the production of reactive oxygen species (ROS)⁴² (Supplementary Fig. 4). PUFAs have a strong affinity or absorption to ROS⁴⁵, therefore, the increment of PUFAs can be interpreted as a defence response against UV-C-generated cellular ROS.

The increase in USFA when compared to SFA in open raceway ponds confirmed the results obtained for lab-scale studies. This may have implications to biodiesel fuel properties that may possess better cold flow properties but maybe more prone to oxidation. Further studies should be carried out to determine whether biosynthesis of nutraceuticals such as eicosapentaenoic acid (EPA) can be manipulated by UV-C radiation. A significant two-fold increase of EPA was detected at laboratory-scale (Supplementary Fig. 3). UV-C treatment also significantly reduced co-cultured microorganisms (Supplementary Fig. 9), which may be advantageous for water recycling and for minimizing contamination during continuous cultivation, an important factor for commercial production. Figure 4a suggests that some lipids are consumed by microalgae after peak accumulation and during harvesting (with or without UV-C), but

final UV-C treatment for settling may reduce lipid degradation as cell mortality increases. The protocol of UV-C lipid induction has been successfully applied on non-flagellate *Chlorella* sp. and *Nannochloropsis* sp., resulting in an increase of total lipid production. This was visualized by Nile red staining. Supplementary Figure 11 shows the effect on UV-C treatment on three other microalgae (one flagellate; *Tetraselmis chui*, and two non-flagellate strains; *Chlorella* sp. BR2 and *Nannochloropsis* sp. BR2). To test whether the LIS protocol also resulted in enhanced settling for other flagellate microalgae, *Dunaliella salina* and *Tetraselmis chui* were subjected to UV-C treatment at different dosages. As shown in Supplementary Figure 12, UV-C treatment also resulted in effective settling for both flagellate microalgae.

In summary, this study used low exposure of UV-C radiation to induce lipids and harvest microalgae at the same time. Outdoor cultivation of *Tetraselmis* sp. M8 in open raceway modules showed that UV-C radiation could be a rapid and effective process for LIS of flagellate microalgae. UV-C radiation could also control co-cultured microorganisms, facilitate water recycling and may assist in subsequent lipid extraction by pretreating cells, all of which could potentially be well integrated in a microalgae biorefinery concept. UV-C-mediated LIS may provide an important step towards commercial microalgal biofuel production.

Acknowledgements

We wish to thank the Australian Research Council for financial support and Drs Skye Thomas-Hall and Simon Tannock for useful discussions.

Figure legends

Figure 1. Nile red-stained cells of *Tetraselmis* sp. M8 that received different doses of UV-C exposure.

a) Cells with maximum lipid fluorescence (yellow) can be observed at 250 mJ and 500 mJ UV-C radiation. Starting from UV-C of 100 mJ/cm² detached flagella can be observed (white arrow), as the radiation is increased to 1000 mJ/cm² cell rupturing occurs (white arrow) with lipid bodies released in the medium. Cells are shown at 40x magnification (bar=50 μ m) at 24 h after treatment. **b)** Kill curve of *Tetraselmis* sp. M8, showing the number of cells that survived UV-C treatment at different dosages. Shown are mean values \pm SEs from three independent treatments of M8 culture. The arrow indicates the LD 50 value.

Figure 2. Lipid induction in *Tetraselmis* sp. M8 cultures at 24 h after receiving different UV-C dosages.

a-c) FACS analysis of Nile red stained cells showing distribution (**a**) and lipid fluorescence (**b**) of low (P1) and high (P2) fluorescence cell populations and of the total population (**c**). **d)** Triacylglyceride quantification by gas chromatography-mass spectroscopy (GC-MS) showing total fatty acids as well as saturated (SFA) and unsaturated (USFA) fatty acids produced by different UV-C-treated cultures. Shown are mean values \pm SEs from three separately-grown and treated microalgae cultures. Bars with different letters indicate significant differences ($P < 0.05$). See Supplementary Figure 1 for the corresponding scatter plots of P1 and P2 populations and Supplementary Figures 2 and 3 for graphs of individual fatty acids of microalgae treated with different UV-C dosages.

Figure 3. Settling of control and UV-C-treated algal culture in 1000-L airlift-mixed raceway ponds. **a)**

Cell counts indicating different phases of the experiment on day 3, 5 and 7. **b)** Settling curve of control and UV-C-treated culture on day 8. **c)** Settling of UV-C-treated and control culture. **d,e)** Settling of UV-C-treated and control culture, respectively, in 1000-L raceway ponds against the background of a white plate. Shown for a-c are mean values \pm SEs from three measurements. The experiment was repeated three times with similar results.

Figure 4. Lipid fluorescence of Nile red-stained cells in control and UV-C-treated raceway ponds.

Maximum lipid fluorescence was observed on day 6 which was an indication of UV-C lipid induction. Shown are total culture lipid fluorescence (**a**) and lipid fluorescence per cell (**b**) as mean values \pm SEs ($n = 3$). Asterisks indicate the time points when UV-C treatment was applied.

Figure 5. Biomass dry weights and fatty acid profiles in harvested culture of control and UV-C-treated raceway-grown algal cultures. Shown are total dry weights (**a**), total fatty acid contents (**b**), as well as individual and total saturated (**c**) and unsaturated (**d**) fatty acid profiles. Bars with (*) show significant differences ($P < 0.05$). Shown are mean values \pm SEs from three separately-grown and -treated raceway cultures.

Supplementary figure legends

Supplementary Figure 1. Fluorescence-Activated Cell Sorting (FACS) analysis of *Tetraselmis* sp. M8

cells that received different UV-C dosages. Shown are unstained Nile red control (a), untreated control stained with Nile red (b) and UV-C treated populations at 100 mJ/cm², 250 mJ/cm², 500 mJ/cm² and 1000 mJ/cm² (c-f; respectively). Signals from unstained control cells were used as a baseline cut-off for the P1 population (red) and cells above this cut-off were marked as the lipid-producing P2 population (green). The Y-axes show fluorescence intensities at a PE excitation wavelength of 575 nm and the X-axes show the forward scatter based on cell size.

Supplementary Figure 2. Comparison of different saturated fatty acids present in *Tetraselmis* sp. M8

microalgae following different dosages of UV-C radiation. Shown are mean values ± SEs from three separately-grown and -treated cultures. Different letters show statistically significant differences ($P < 0.05$).

Supplementary Figure 3. Comparison of different unsaturated fatty acids present in *Tetraselmis* sp.

M8 microalgae following different dosages of UV-C radiation. Shown are mean values ± SEs from three separately-grown and -treated cultures. Different letters show statistically significant differences ($P < 0.05$).

Supplementary Figure 4. Enzymatic assays of UV-C-stressed and mock-treated *Tetraselmis* sp. M8

cells at different dosages. a) Decomposition of unstable peroxides (TBRAS) assay, b) Superoxide dismutase (SOD) assay, c) Glutathione reductase (GR) assay (normalized to cell count). Values are mean ± SEs from three separately-grown and -treated cultures.

Supplementary Figure 5. Equipment for small-scale outdoor microalgae UV-C treatment.

Shown are the design of the UV-C chamber (a-d) and the experimental setup of the UV-C treatment for microalgae in 20 L cultures (e). The UV-C chamber delivered approximately 12 J cm⁻² h⁻¹.

Supplementary Figure 6. Timecourse analysis of *Tetraselmis* sp. M8 culture settling following UV-C

treatment. Shown are cell counts in the medium from three independent 20 L outdoor cultures with a depth of 20 cm and UV-C exposure of 3 J/cm².

Supplementary Figure 7. Photograph of two identically designed 1000 L-raceway ponds whose cultures were aerated and circulated by airlifts with diagonally-applied pressured air.

Supplementary Figure 8: Lipid productivity of *Tetraselmis* sp. M8 culture following UV-C treatment. Bars with (*) show significant differences ($P < 0.05$). Shown are mean values \pm SEs from three separately-grown and -treated outdoor raceway cultures.

Supplementary Figure 9. UV-C treatment reduces microbial presence in algal cultures. a,b) LB and PDB plates, respectively, with UV-C-treated used algae culturing medium (left plate) showing significantly fewer bacterial and fungal colonies, respectively, when compared to the mock-treated control (right plate). **c,d)** Quantification of culturable bacterial (c) and fungal (d) colonies shown as mean values with SEs from three separately-grown and -treated raceway algal cultures.

Supplementary Figure 10. Effect of low dosage of UV-C radiation on the fatty acid synthesis pathway. UV-C radiation might help in conversion of saturated fatty acids that serve as a storage function in plastids to unsaturated fatty acids that may reduce oxidative cell damage. Shown are total and percentage increases in pie charts for each fatty acid.

Supplementary Figure 11. Increase in lipid fluorescence (yellow) before and after UV-C radiation in different microalgae. a,b) Control and UV-C treated (100 mJ) *Chlorella* sp. BR2; **c,d)** Control and UV-C treated (100 mJ) *Tetraselmis chui*; **e,f)** Control and UV-C treated (250 mJ) *Nannochloropsis* sp. BR2. Photographs were taken of Nile red-stained microalgae at 40x magnification (bar=50 μ m) at 24 h after treatment.

Supplementary Figure 12. Induced settling of microalgae at different dosages of UV-C radiation at 6 hours after treatment: a) *Dunaliella salina*; **b)** *Tetraselmis chui*. Shown are mean values with SEs from three separately-grown and -treated 50 mL algal cultures.

Table 1. Costs of harvesting 10,000 L of *Tetraselmis* sp. culture with different harvesting techniques.

	Single Step Dewatering	Primary Dewatering			Secondary Dewatering	
	Centrifugation	Sedimentation	Flotation	LIS UV-C	Filtration	Centrifugation
Total Energy Consumed	55 kWh/10 m ³ [^]	-	7.4-8.4 kWh/10 m ³ ⁺	5.76 kWh/10 m ³	1-3 kWh/m ³	5.5 kWh/m ³ [^]
Time Required (hours)	10	-	2	6	1	1
Energy Cost (AUD)[§]	\$12.10*	-	\$1.62 – \$1.84*	\$1.26*	\$0.22 – \$0.66*	\$1.21*
Dosage required	-	400 g @ 40 mg L ⁻¹ ₄₆	80 g @ 8 mg L ⁻¹ ⁴⁷		-	-
Chemicals (AUD)	-	\$10 (Chitosan @ \$25/kg)	\$0.64 (CTAB @ \$8/kg)	-	-	-
pH Adjustment Dosage	-	1.5 to 2 L acetic acid~	-	-	-	-
pH Adjustment Cost	-	\$1.20 – \$1.60 @ \$800/t	-	-	-	-
Total Costs (AUD)	\$12.10	\$11.10 - 11.20	\$2.26 – \$2.28	\$1.26	\$0.22 – \$0.66	\$1.21

[§] Australian Dollar (= approx. US \$1.04)

* Electricity prices were calculated based on \$0.22 per kWh

[^] An Evodos (E-25) centrifuge was used for this study (at the flow rate of 1000 L/h).

⁺ The flotation cell considered is a Jameson cell and energy consumption was determined using various published studies ⁴⁷.

[~] The volume was estimated by doing an experiment with 1 L of algae culture and mathematical calculation.

Values may differ for different algal strains as these may have different preferential harvesting conditions.

References

1. R. H. Wijffels and M. J. Barbosa, *Science*, 2010, **329**, 796-799.
2. F. X. Malcata, *Trends Biotech.*, 2011, **29**, 542-549.
3. C. Wilhelm and T. Jakob, *Appl. Microbiol. Biotech.*, 2011, **92**, 909-919.
4. P. Schenk, S. Thomas-Hall, E. Stephens, U. Marx, J. Mussgnug, C. Posten, O. Kruse and B. Hankamer *BioEnerg. Res.*, 2008, **1**, 20-43.
5. Y. Chisti, *J. Biotechnology.*, 2013, **167**, 201-214.
6. L. Brennan and P. Owende, *Renew. Sustain. Energ. Rev.*, 2010, **14**, 557-577.
7. E. Stephens, I. L. Ross, J. H. Mussgnug, L. D. Wagner, M. A. Borowitzka, C. Posten, O. Kruse and B. Hankamer, *Trends Plant Sci.*, 2010, **15**, 554-564.
8. P. McGinn, K. Dickinson, S. Bhatti, J.-C. Frigon, S. Guiot and S. O'Leary, *Photosynth. Res.*, 2011, **109**, 231-247.
9. K. K. Sharma, H. Schuhmann and P. M. Schenk, *Energies*, 2012, **5**, 1532-1553.
10. B. Wang, Y. Li, N. Wu and C. Q. Lan, *Appl. Microbiol. Biotech.*, 2008, **79**, 707-718.
11. K. K. Sharma, S. Garg, Y. Li, A. Malekizadeh and P. M. Schenk, *Biofuels*, 2013, **4**, 397-407.
12. A. Kumar, S. Ergas, X. Yuan, A. Sahu, Q. Zhang, J. Dewulf, F. X. Malcata and H. Van Langenhove, *Trends Biotechnol.*, 2010, **28**, 371-380.
13. J. K. Pittman, A. P. Dean and O. Osundeko, *Bioresource Tech.*, 2011, **102**, 17-25.
14. E. Molina Grima, E. H. Belarbi, F. G. Acién Fernández, A. Robles Medina and Y. Chisti, *Biotech. Adv.*, 2003, **20**, 491-515.
15. S. A. Khan, Rashmi, M. Z. Hussain, S. Prasad and U. C. Banerjee, *Renew. Sustain. Energ. Rev.*, 2009, **13**, 2361-2372.
16. X. Zeng, M. K. Danquah, X. D. Chen and Y. Lu, *Renew. Sustain. Energ. Rev.*, 2011, **15**, 3252-3260.
17. T. C. Guadin C, *Adv. Biotechnol. Process.*, 1986, **6**, 73-110.
18. T. M. Mata, A. A. Martins and N. S. Caetano *Renew. Sustain. Energ. Rev.*, 2010, **14**, 217-232.
19. A. Singh, P. S. Nigam and J. D. Murphy, *Bioresource Tech.*, 2011, **102**, 26-34.
20. J. Yang, M. Xu, X. Zhang, Q. Hu, M. Sommerfeld and Y. Chen, *Bioresource Tech.*, 2011, **102**, 159-165.
21. S. M. Renaud, L.-V. Thinh, G. Lambrinidis and D. L. Parry, *Aquaculture*, 2002, **211**, 195-214.
22. R. Srinivas and C. Ochs, *Photochem. Photobiol.*, 2012, **88**, 684-689 .
23. B. Riaño, B. Molinuevo and M. C. García-González *Ecol. Eng.*, 2012, **38**, 110-113.
24. J. I. Goes, N. Handa, S. Taguchi, T. Hama and H. Saito, *J. Plankton Res.*, 1995, **17**, 1337-1362.
25. L. Y. Zhu, X. C. Zhang, L. Ji, X. J. Song and C. H. Kuang, *Process Biochem.*, 2007, **42**, 210-214.
26. Z. Xue, P. Sharpe, S.-P. Hong, N. Yadav, D. Xie, D. Short, H. Damude, R. Rupert, J. Seip, J. Wang, D. Pollak, M. Bostick, M. Bosak, D. Macool, D. Hollerbach, H. Zhang, D. Arcilla, S. Bledsoe, K. Croker, E. McCord, B. Tyreus, E. Jackson and Q. Zhu, *Nat. Biotech.*, 2013, **31**, 734-740
27. A. Widjaja, C.-C. Chien and Y.-H. Ju, *J. Taiwan Inst. Chem. Engineers.*, 2009, **40**, 13-20.
28. W. L. Yu, W. Ansari, N. G. Schoepp, M. J. Hannon, S. P. Mayfield and M. D. Burkart, *Microb. Cell. Fact.*, 2011, **10**, 91.

29. Y. Jiang and F. Chen, *J. Ind. Microbiol. Biotechnol.*, 1999, **23**, 508-513.
30. Y. Liang, J. Beardall and P. Heraud, *J. Photochem. Photobiol. B: Biology.*, 2006, **82**, 161-172.
31. E. Forján, I. Garbayo, M. Henriques, J. Rocha, J. Vega and C. Vílchez, *Marine Biotech.*, 2011, **13**, 366-375.
32. D. K. Y. Lim, S. Garg, M. Timmins, E. S. B. Zhang, S. R. Thomas-Hall, H. Schuhmann, Y. Li and P. M. Schenk, *PLoS ONE*, 2012, **7**, e40751.
33. M. J. Dring, V. Makarov, E. Schoschina, M. Lorenz and K. Lüning, *Marine Biol.*, 1996, **126**, 183-191.
34. D. Karentz, J. E. Cleaver and D. L. Mitchell, *J. Phycology.*, 1991, **27**, 326-341.
35. M. Chen, H. Tang, H. Ma, T. C. Holland, K. Y. S. Ng and S. O. Salley, *Bioresource Tech.*, 2011, **102**, 1649-1655.
36. F. Guihéneuf, M. Fouqueray, V. Mimouni, L. Ulmann, B. Jacquette and G. Tremblin, *J. Appl. Phycol.*, 2010, **22**, 629-638.
37. L. J. Rothschild, *J. Eukaryot. Microbiol.*, 1999, **46**, 548-555.
38. C. S. Cockell, *Planet.Space Sci.*, 2000, **48**, 203-214.
39. C. S. Cockell and J. A. Raven, *Philosoph. Transactions Roy. Soc. A: Math., Physic. Eng. Sci.*, 2007, **365**, 1889-1901.
40. Z.-Y. Liu, G.-C. Wang and B.-C. Zhou, *Bioresource Tech.*, 2008, **99**, 4717-4722.
41. M. Takagi, Karseno and T. Yoshida, *J. Biosci. Bioeng.*, 2006, **101**, 223-226.
42. R. Gupta, P. Bhadauriya, V. Chauhan and P. Bisen, *Curr. Microbiol.*, 2008, **56**, 156-161.
43. M. Brett and D. Müller-Navarra, *Freshwater Biol.*, 1997, **38**, 483-499.
44. X. Chi, X. Zhang, X. Guan, L. Ding, Y. Li, M. Wang, H. Lin and S. Qin, *J. Microbiol.*, 2008, **46**, 189-201.
45. R. Bouhamidi, V. Prévost and A. Nouvelot, *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie*, 1998, **321**, 31-38.
46. M. Heasman, J. Diemar, W. O'Connor, T. Sushames and L. Foulkes, *Aquaculture Res.*, 2000, **31**, 637-659.
47. S. Garg, Y. Li, L. Wang and P. M. Schenk, *Bioresource Tech.*, 2012, **121**, 471-474.
48. Guillard, RR & Ryther, JH *J. Microbiol.*, 1962, **196**, 28:229-239.

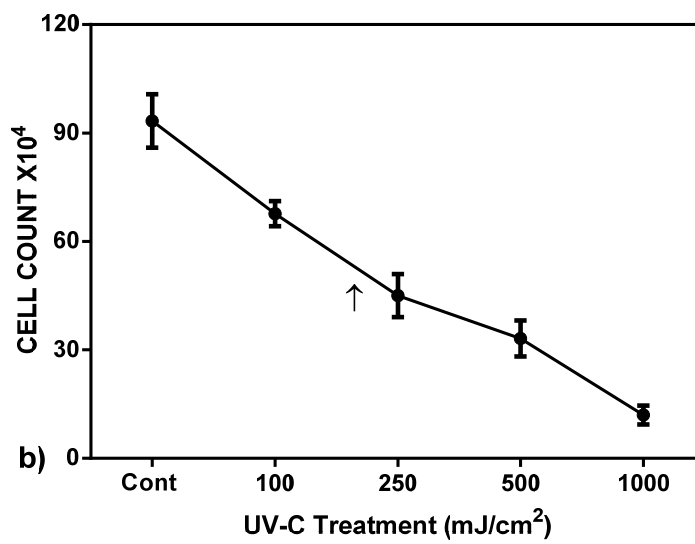
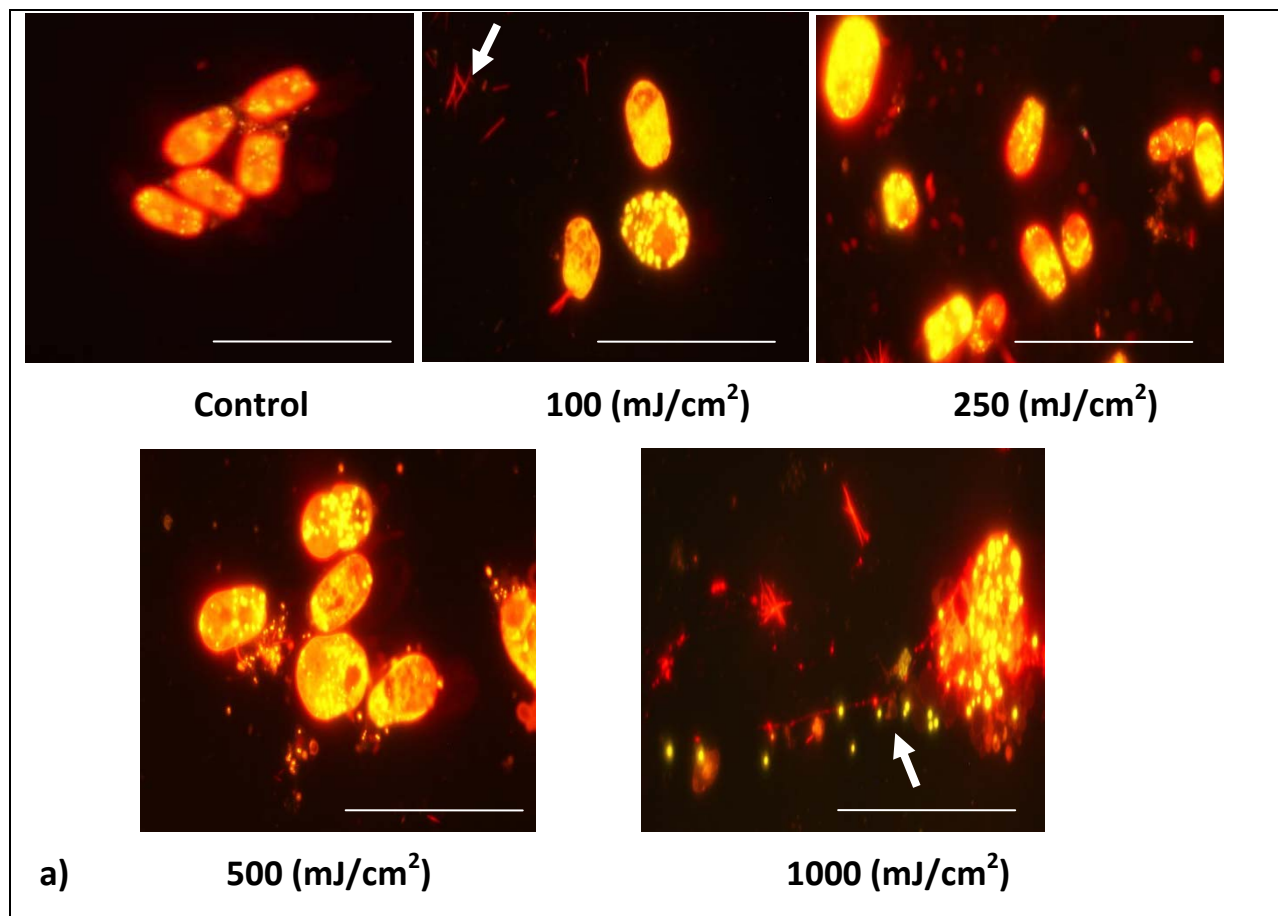


Figure 1.

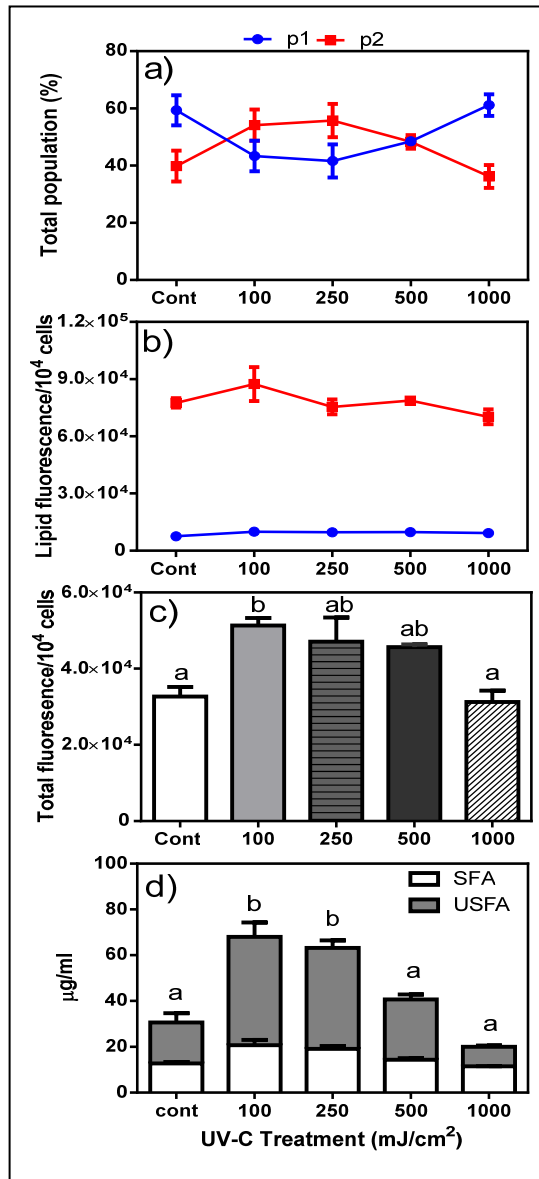


Figure 2.

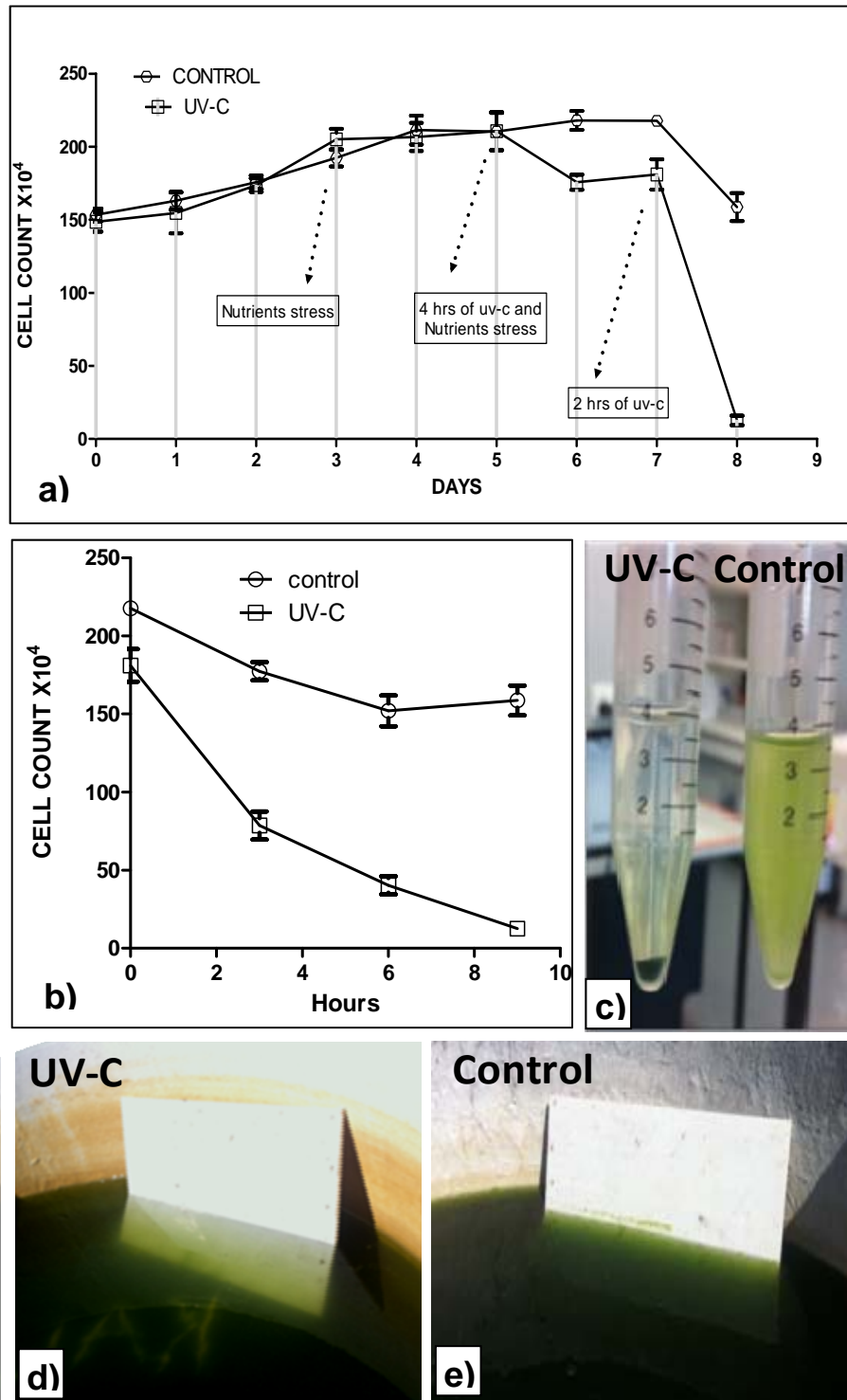


Figure 3.

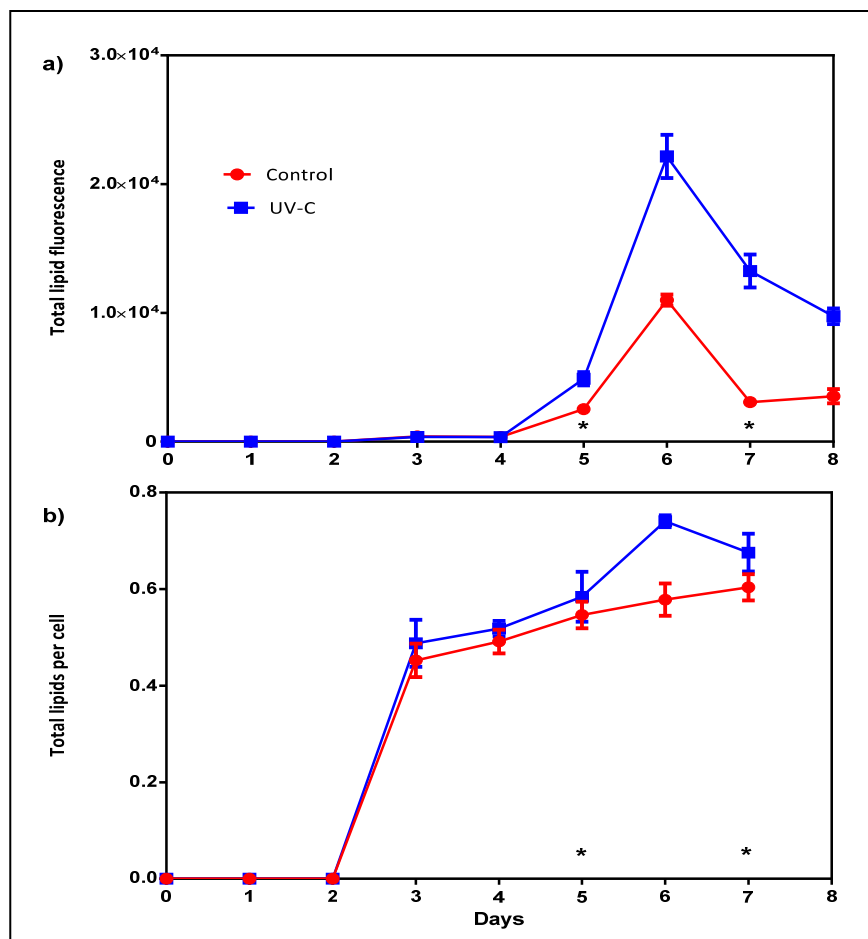


Figure 4.

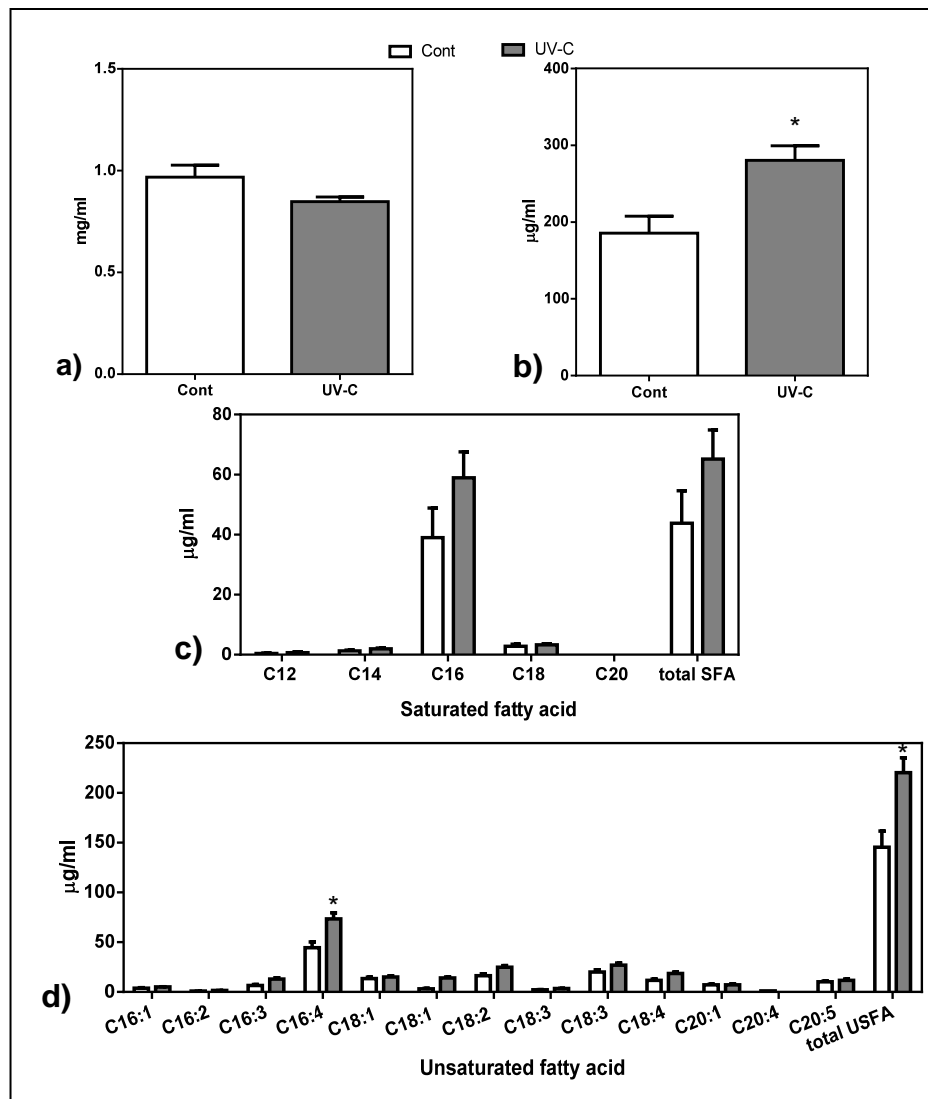
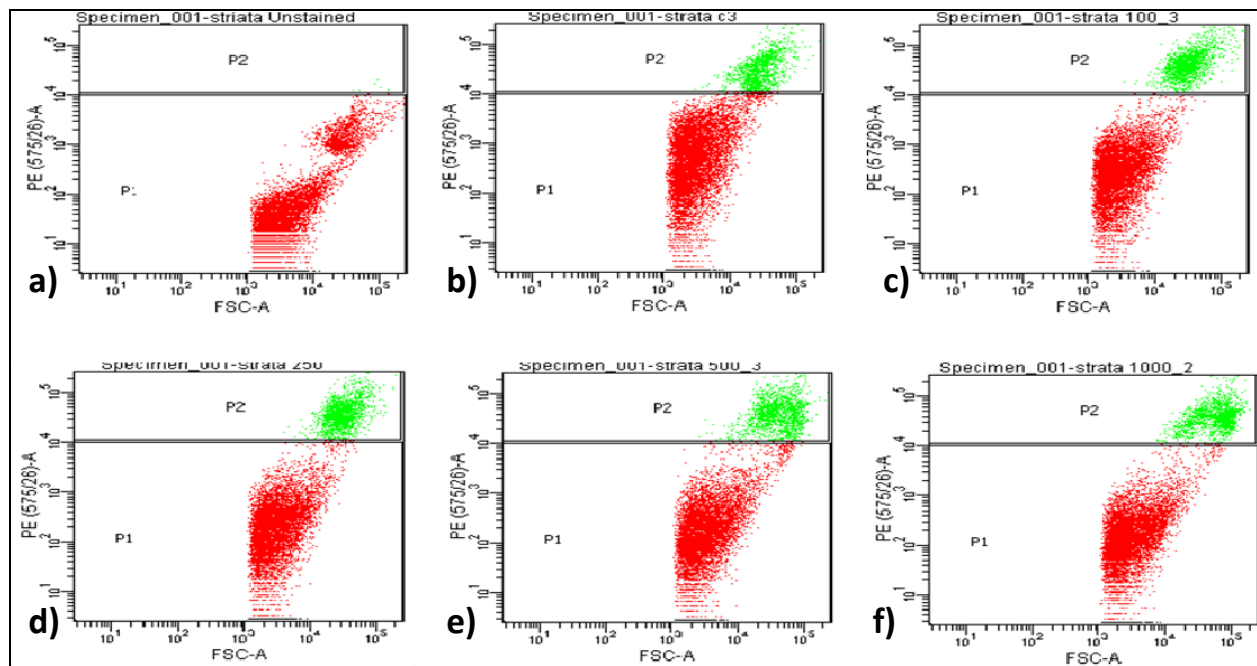
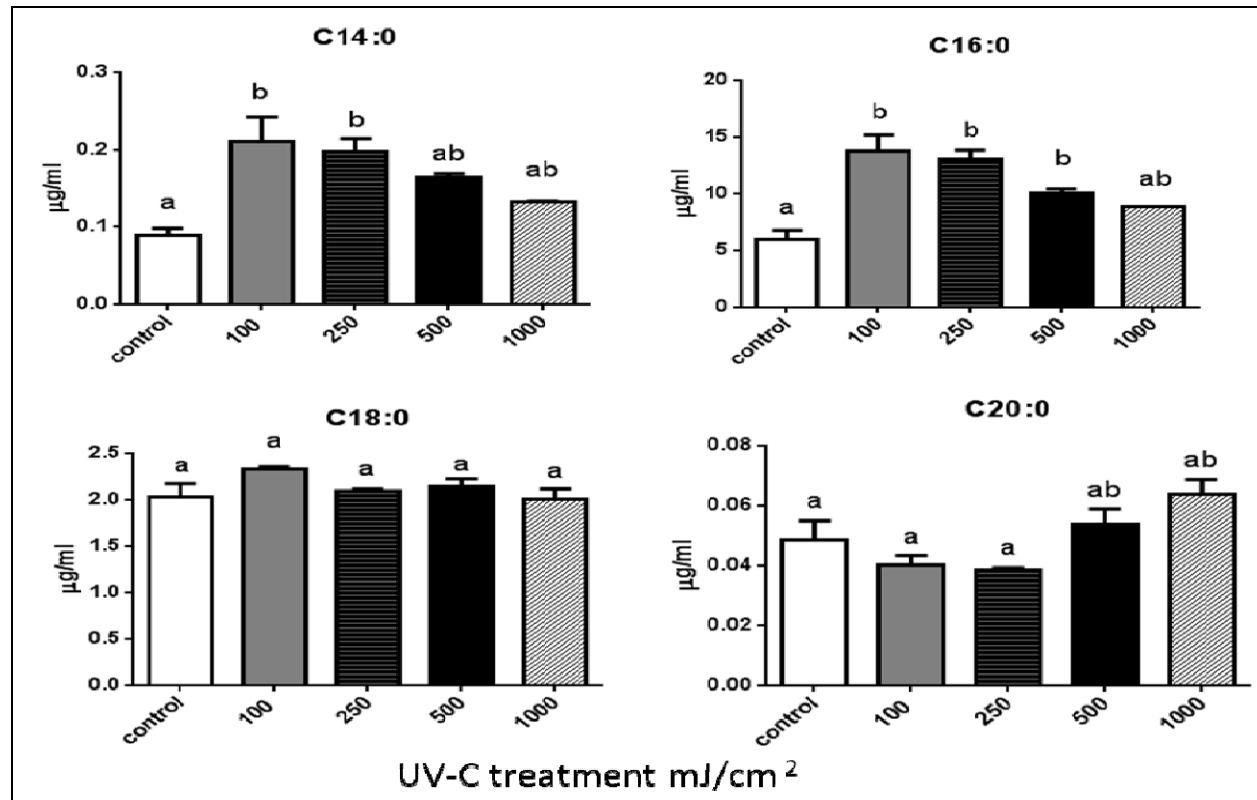


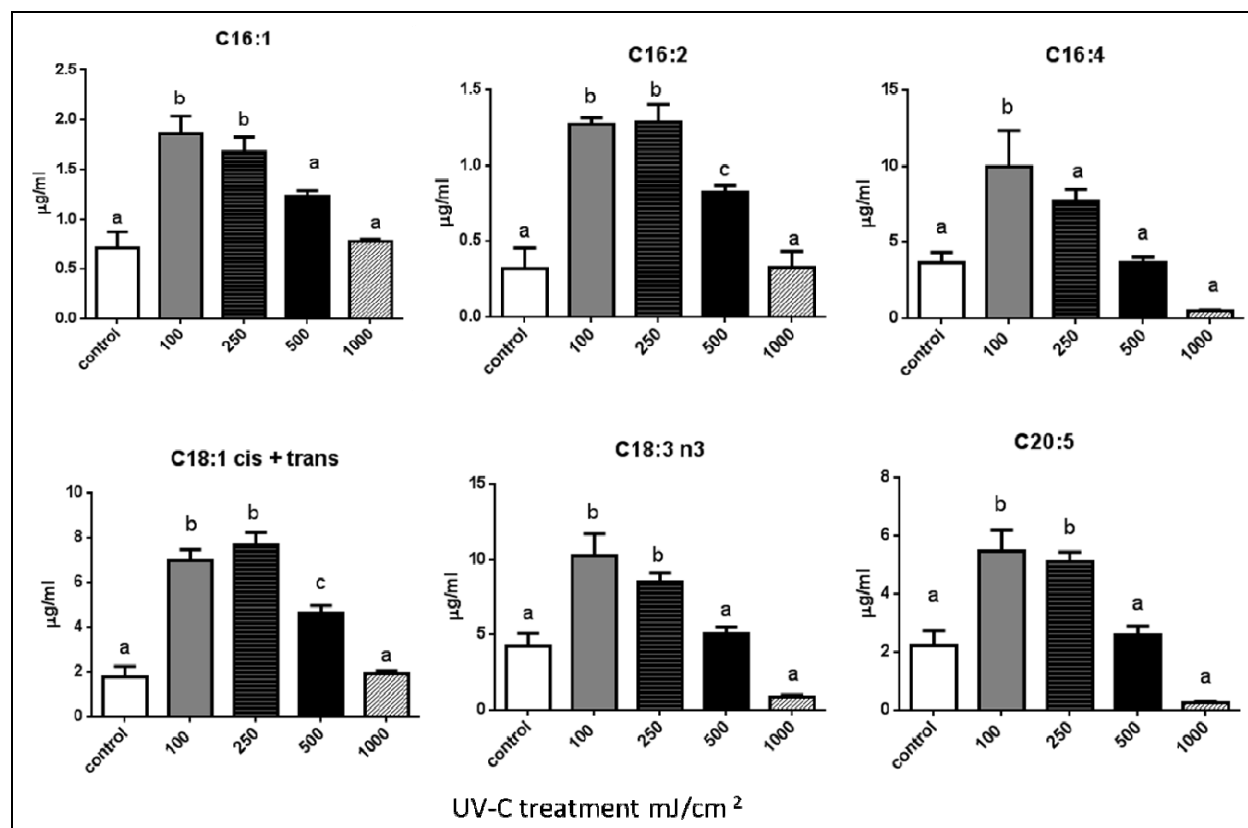
Figure 5.



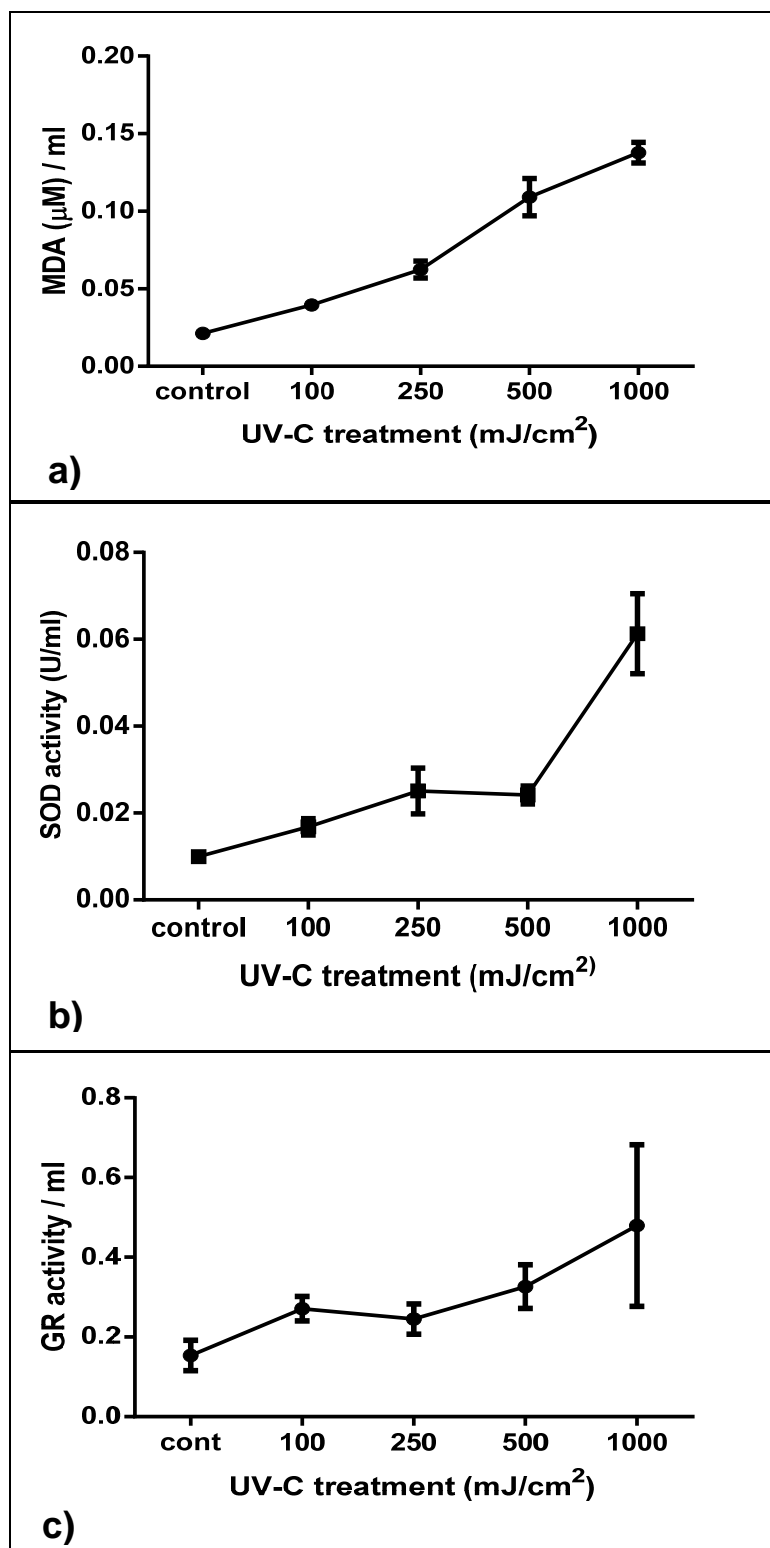
Supplementary Figure 1. Fluorescence-Activated Cell Sorting (FACS) analysis of *Tetraselmis* sp. M8 cells that received different UV-C dosages. Shown are unstained Nile red control (a), untreated control stained with Nile red (b) and UV-C treated populations at 100 mJ/cm², 250 mJ/cm², 500 mJ/cm² and 1000 mJ/cm² (c-f; respectively). Signals from unstained control cells were used as a baseline cut-off for the P1 population (red) and cells above this cut-off were marked the as lipid-producing P2 population (green). The Y-axes show fluorescence intensities at a PE excitation wavelength of 575 nm and the X-axes show the forward scatter based on cell size.



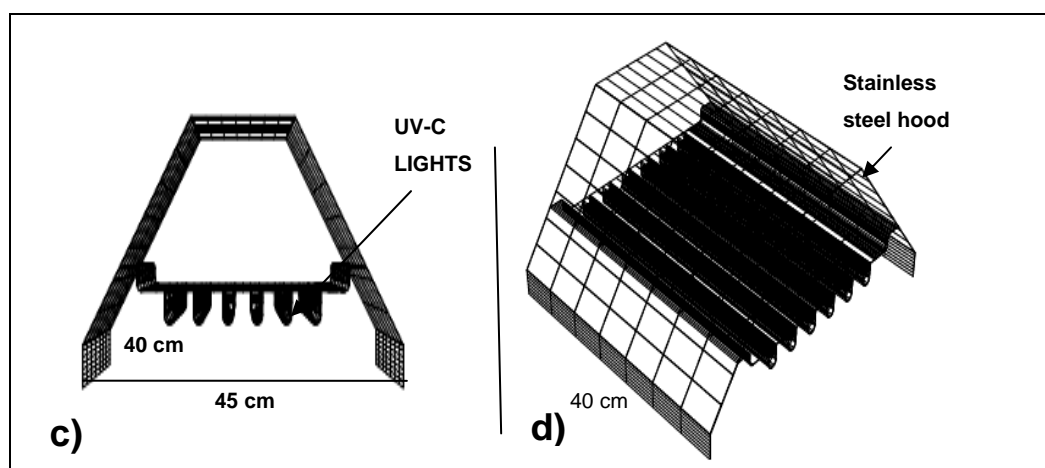
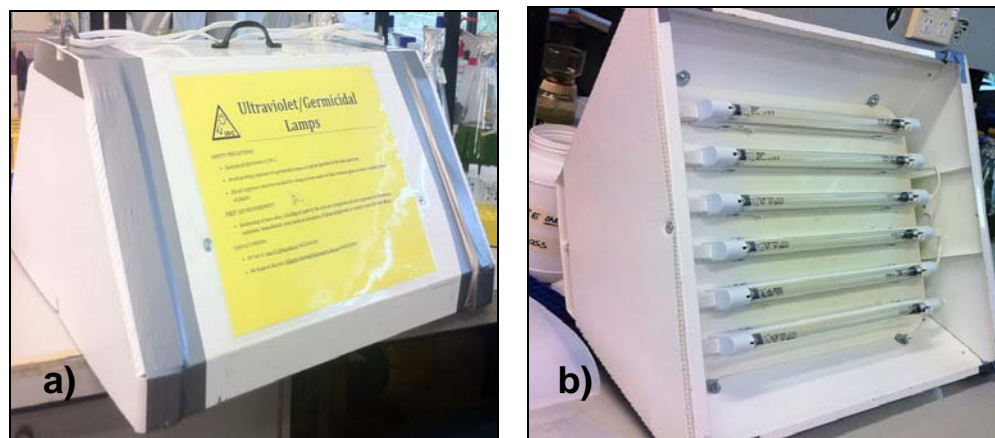
Supplementary Figure 2. Comparison of different saturated fatty acids present in *Tetraselmis* sp. M8 microalgae following different dosages of UV-C radiation. Shown are mean values \pm SEs from three separately-grown and -treated cultures. Different letters show statistically significant differences ($P < 0.05$).



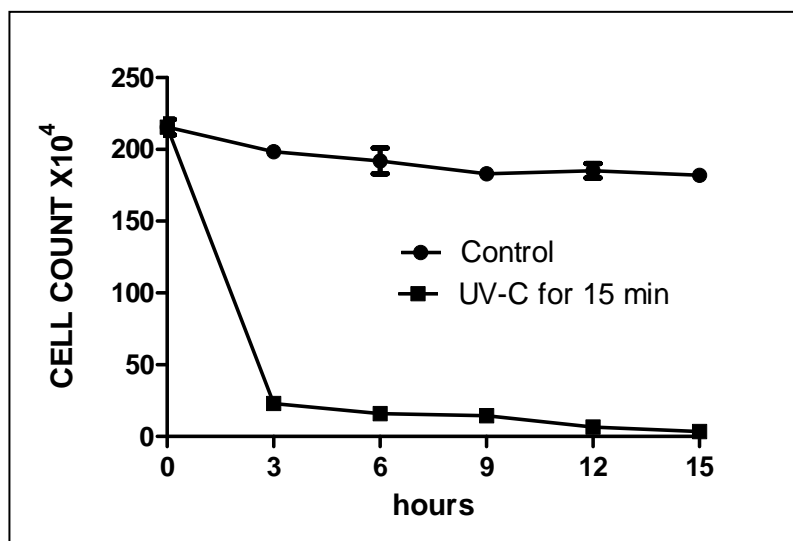
Supplementary Figure 3. Comparison of different unsaturated fatty acids present in *Tetraselmis* sp. M8 microalgae following different dosages of UV-C radiation. Shown are mean values \pm SEs from three separately-grown and -treated cultures. Different letters show statistically significant differences ($P < 0.05$).



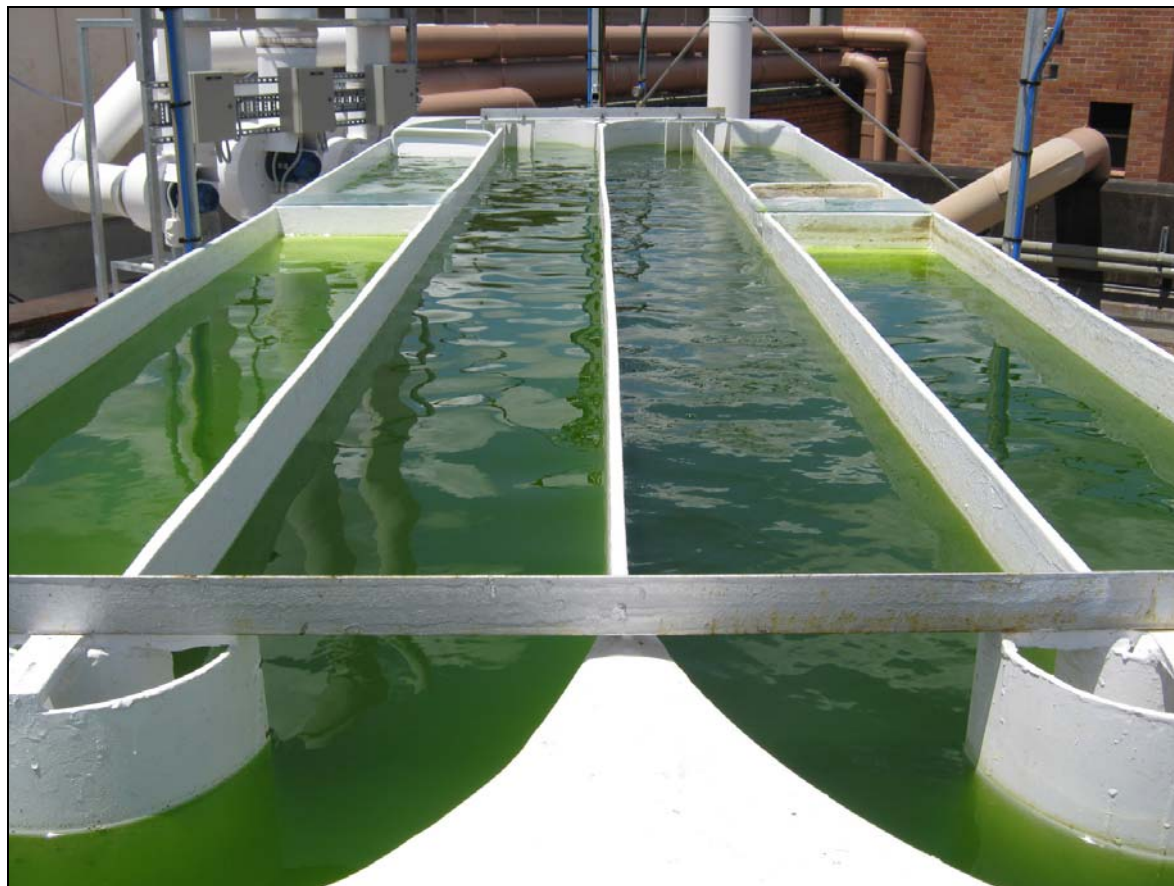
Supplementary Figure 4. Enzymatic assays of UV-C-stressed and mock-treated *Tetraselmis* sp. M8 cells at different dosages. **a)** Decomposition of unstable peroxides (TBRAS) assay, **b)** Superoxide dismutase (SOD) assay, **c)** Glutathione reductase (GR) assay (normalized to cell count). Values are mean \pm SEs from three separately-grown and -treated cultures.



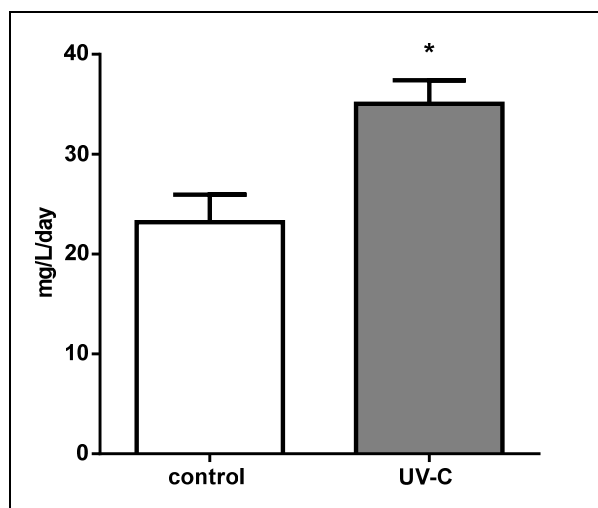
Supplementary Figure 5. Equipment for small-scale outdoor microalgae UV-C treatment. Shown are the design of the UV-C chamber (a-d) and the experimental setup of the UV-C treatment for microalgae in 20 L cultures (e). The UV-C chamber delivered approximately $12 \text{ J cm}^{-2} \text{ h}^{-1}$.



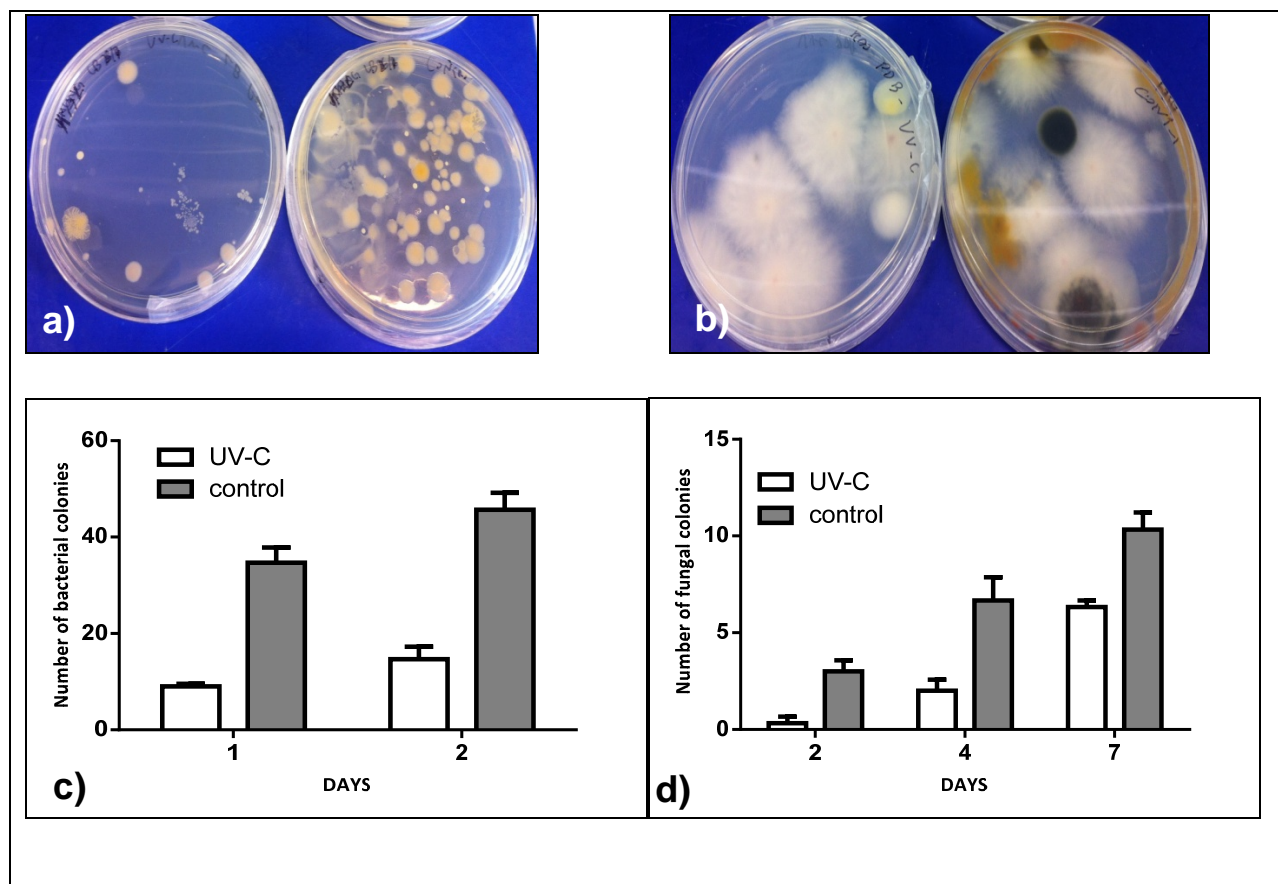
Supplementary Figure 6. Timecourse analysis of *Tetraselmis* sp. M8 culture settling following UV-C treatment. Shown are cell counts in the medium from three independent 20 L outdoor cultures with a depth of 20 cm and UV-C exposure of 3 J/cm².



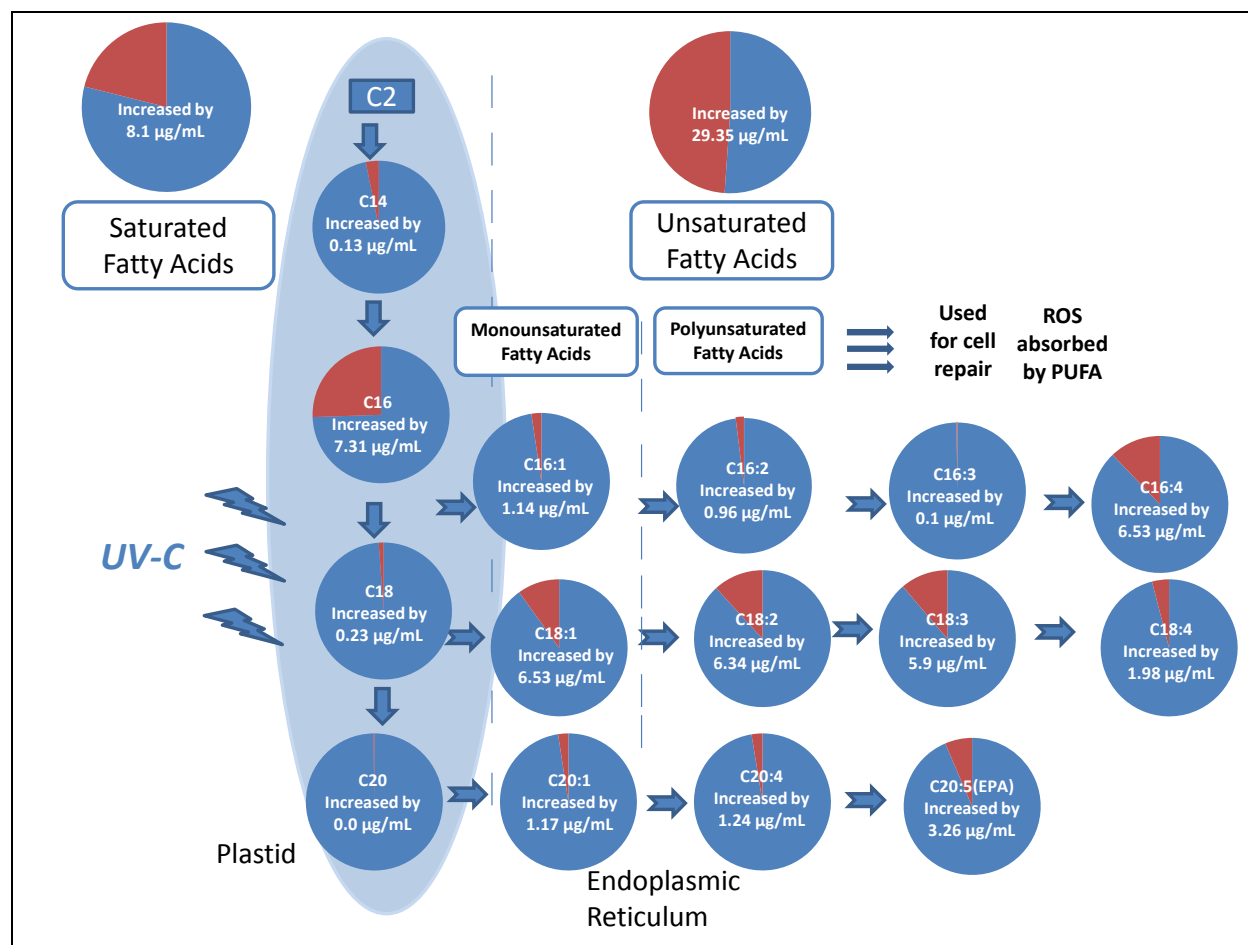
Supplementary Figure 7. Photograph of two identically designed 1000 L-raceway ponds whose cultures were aerated and circulated by airlifts with diagonally-applied pressured air.



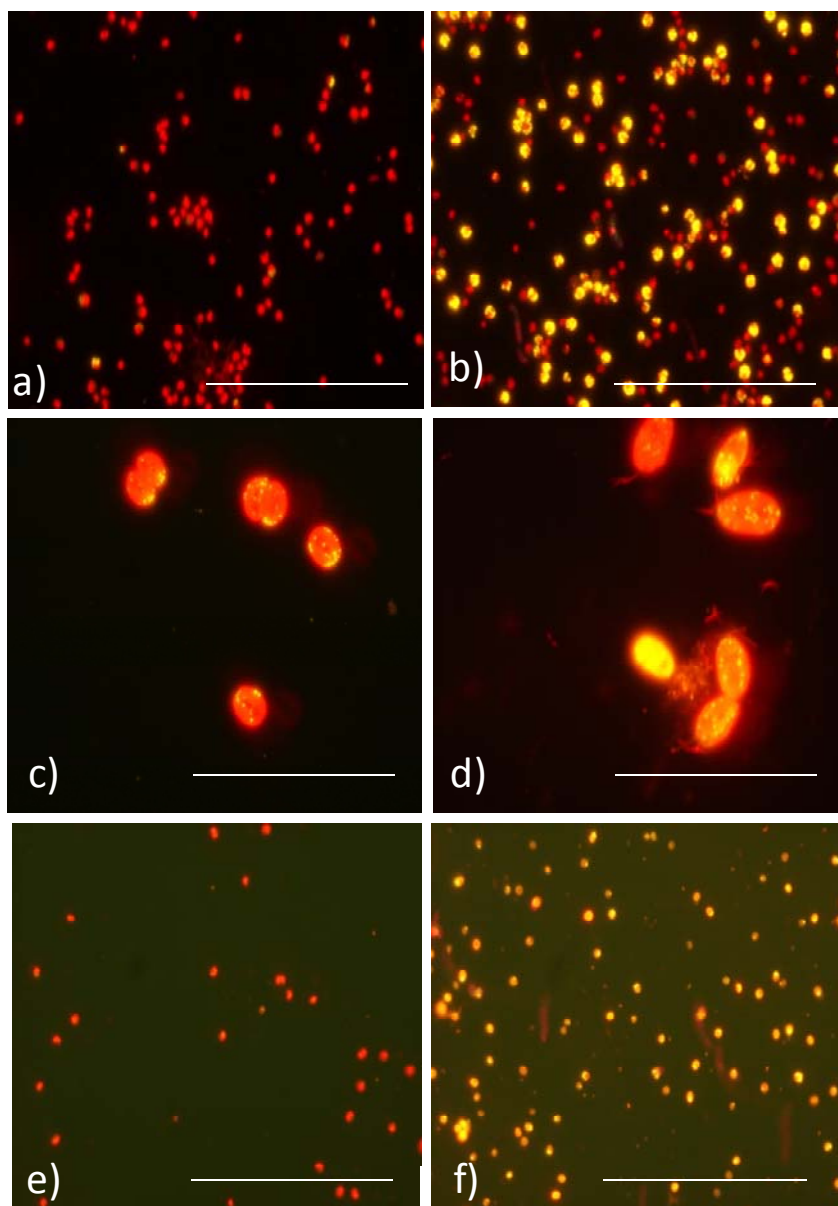
Supplementary Figure 8: Lipid productivity of *Tetraselmis* sp. M8 culture following UV-C treatment. Bars with (*) show significant differences ($P < 0.05$). Shown are mean values \pm SEs from three separately-grown and -treated outdoor raceway cultures.



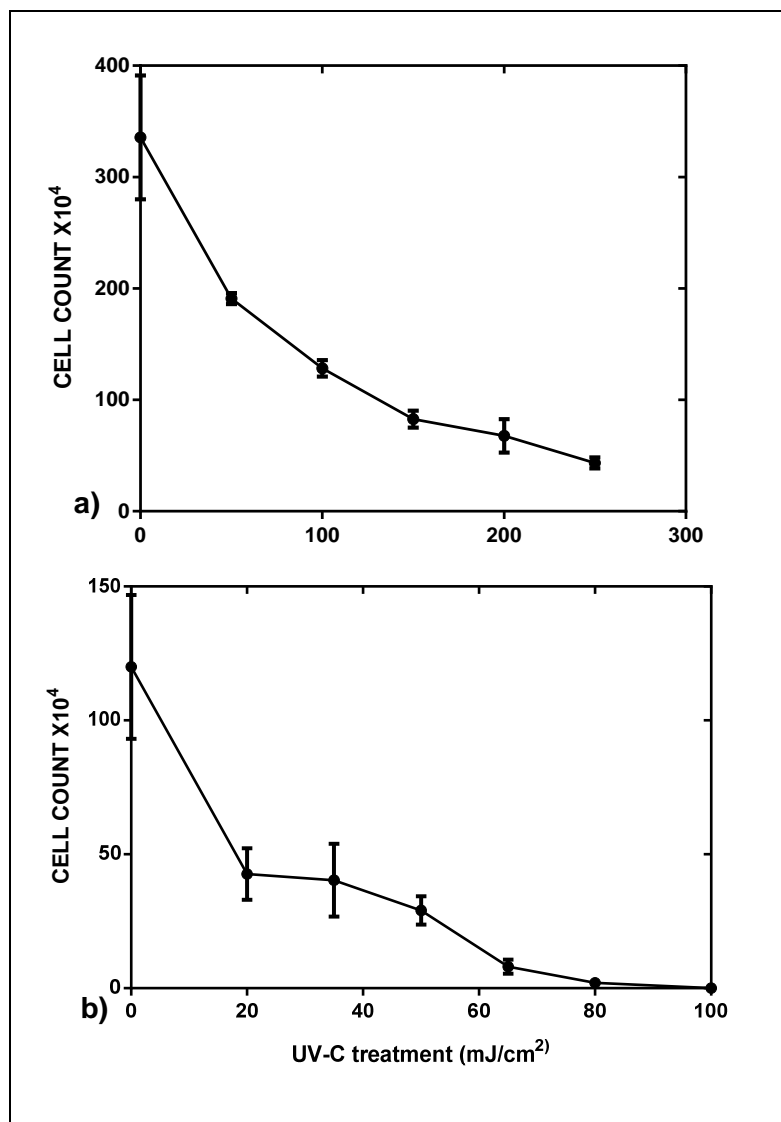
Supplementary Figure 9. UV-C treatment reduces microbial presence in algal cultures. **a,b)** LB and PDB plates, respectively, with UV-C-treated used algae culturing medium (left plate) showing significantly fewer bacterial and fungal colonies, respectively, when compared to the mock-treated control (right plate). **c,d)** Quantification of culturable bacterial (c) and fungal (d) colonies shown as mean values with SEs from three separately-grown and -treated raceway algal cultures.



Supplementary Figure 10. Effect of low dosage of UV-C radiation on the fatty acid synthesis pathway. UV-C radiation might help in conversion of saturated fatty acids that serve as a storage function in plastids to unsaturated fatty acids that may reduce oxidative cell damage. Shown are total and percentage increases in pie charts for each fatty acid.



Supplementary Figure 11. Increase in lipid fluorescence (yellow) before and after UV-C radiation in different microalgae. a,b) Control and UV-C treated (100 mJ) *Chlorella* sp. BR2; **c,d)** Control and UV-C treated (100 mJ) *Tetraselmis chui*; **e,f)** Control and UV-C treated (250 mJ) *Nannochloropsis* sp. BR2. Photographs were taken of Nile red-stained microalgae at 40x magnification (bar=50 μm) at 24 h after treatment.



Supplementary Figure 12. Induced settling of microalgae at different dosages of UV-C radiation at 6 hours after treatment: a) *Dunaniella salina*; b) *Tetraselmis chui*. Shown are mean values with SEs from three separately-grown and -treated 50 mL algal cultures.