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Evaluation of growth, cell size and biomass of *Isochrysis* aff. *galbana* (T-ISO) with two LED regimes

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Abstract

In contrast to crops, there are few studies using LED-based light with microalgae and none cultivating the microalga *Isochrysis* aff. *galbana* (T-ISO) despite its importance to marine aquaculture. The objective was to evaluate white and red LEDs as an alternative source of light for the cultivation of *I.* aff. *galbana* (T-ISO). In order to carry this out**,** white and red LEDs were used with a laboratory built Erlenmeyer-type photobioreactor to determine productivity, cell number**,** size and biomass composition. Results were compared with standard fluorescent lights of the same light intensity. The culture system consisted of 3 flasks for applying red LEDs**,** 3 flasks for white LEDs and 3 control group flasks illuminated with the normal fluorescent lighting at the similar light intensity of ~60 μM m^{-2} s^{-1}. It was found that the population cell density did not significantly increase with either red LEDs or white LEDs ($p > 0.05$), if at all. Standard fluorescent lighting (control group) showed significant increases in population cell number ($p < 0.05$). Through microscopic observation cell size was found to be smaller for white LEDS**,** and even smaller for red LEDs compared to fluorescent lighting. The biochemical composition of proteins, carbohydrates and lipids was similar for all light regimes. The authors suggest that the unexpected non-growth of *I.* aff. *galbana* (T-ISO), a haptophyte microalga with white and red LEDs**,** is possibly due to the fact that the cell growth initiation of this microalgae requires other wavelengths (possibly green) aside from red and blue, to allow auxiliary pigments, probably fucoxanthin, to capture light.

Keywords: *microalgae, Isochrysis galbana, LEDs*

INTRODUCTION

Marine microalgae are recognized as a rich source of pigments**:** β-carotene, long-chain polyunsaturated fatty acids (PUFAs)**,** polysaccharides and vitamins (1, 2). Although microalgae cultivation is now over 40 years old, it is still a pertinent practice today. Although it was established over 10 years ago, most of the microalgal species being grown commercially on a large scale are still *Spirulina* spp. and *Chlorella* spp. for health food (3)**,** along with a handful of other species principally used in aquaculture as live food for farmed species (4).

Although microalgae biomass is still primarily commercially produced for human consumption and feedstuff in aquaculture, 25–30 years ago after the oil crises of the 1970s the United States Department of Energy (DOE) started the Aquatic Species Program from 1978 to 1996 looking into using microalgae as a biofuel (5). However, due to the low prices of oil at that time the program was discontinued in 1996. Recently, escalating oil prices (> $100/barrel) due to depleting resources and high demand have revived interest in microalgae as a sustainable energy alternative (6, 7). Microalgae are particularly attractive since they can lessen CO_2 emission, they are a nonfood crop, can produce oil with

low land usage (8) in saline or brackish or even wastewater (9) with high productivity and high yields since many strains contain up to 20-80% of their weight in triglycerides convertible into biodiesel (10, 11). For these reasons, there are currently many private companies actively attempting to commercialize fuel derived from microalgae—however, none as far as we are aware are in production.

In addition, due to declining fisheries worldwide, aquaculture activity is on the rise thereby fueling demand for live microalgae (12, 13). This calls for the development of more sophisticated methods of microalgae cultivation, with higher productivity and lower contamination than that available by open ponds or raceways (14, 15). Debates vary, but one general consensus is that ideally production should be in photobioreactors (PBRs) where growth can be better controlled; however, the cost of production has been a looming concern.

One of the principal advantages of closed PBRs, as compared to open ponds or raceways, is that the light path length can be reduced leading to higher cell densities, which also diminishes the possibility of contamination thereby reducing harvesting costs. The development of the light emitting diode (LED) technology over the years and its decrease in the cost of production has led to an increasing number of new applications of LEDs as a light source. LEDs offer a number of advantages over other light technologies such as low cost, longevity**,** low power consumption, low heat generation, and wavelength control.

There are a limited number of studies utilizing high efficiency light-emitting diodes (LEDs) with microalgae, even though LEDs have been proposed as a primary light source in commercial crop cultures due to their lower energy costs compared to standard lighting (16). For example, Matthijs et al., (17) reported that monochromatic exposure from red LEDs alone can support microalgae growth, whereas limited exposure to blue light failed to augment the biomass production

of *Chlorella* sp. However, in that study the effect of blue LEDs alone on the growth of *Chlorella* sp. was not studied and the ratio of blue to red LEDs used may have possibly been too low to cause any effect. In another study, Lee and Palsson (18) reported also that in *Chlorella* sp. red LEDs also had produced equivalent biomass growth in comparison to fluorescent light. Oh et al. (19) studied the effects of irradiance with various wavelengths from light-emitting diodes on the growth of the harmful dinoflagellate *Heterocapsa circularisquama* and the diatom *Skeletonema costatum*; they reported light selectivity in stimulating the growth of one over the other. Wang et al., (20) reported that red light-emitting diodes performed better than blue LEDs in the cultivation of *Spirulina platensis*.

Isochrysis aff. *galbana* (T-ISO) is one of the most commonly used microalgal species in aquaculture (14). *Isochrysis* sp. are small (4–6 μm in diameter), motile with flagella, and since they a lack a cell wall (naked) they are readily digested by small (larval) invertebrates (21). They also have a high content of the essential fatty acid docosahexaenoic acid (DHA, 22:6 (n-3) (22). *Isochrysis* aff. *galbana* are considered an excellent candidate for mass cultivation (23).

Most of the previous studies with *Isochrysis* sp. have been related to light intensity and microalgal growth and not with light quality (24, 25). For the first time, the present study applies both white and red LEDs in a small Erlenmeyer-type bioreactor to determine the cell density, productivity, size and biomass composition of *Isochrysis* aff. *galbana* (T-ISO) and compares the result**s** with standard fluorescent lights. The hypothesis being tested is whether this microalgae grows with red and white LEDS.

MATERIALS AND METHODS

Microalgae Strain and culture conditions The microalga *Isochrysis* aff. *galbana* (T-ISO; UTEX LB 2307) from the Culture Collection of Microalgae at the Centro de Investigaciones Biológicas del Noroeste S.C., La Paz, B.C.S., Mexico. The initial microalgae inoculums were grown in three 2-L Erlenmeyer flasks in sterilized seawater (≈ 34 PSU) with f/2 medium (26) with continuous air bubbling (1 vvm). Full spectrum white light was supplied continuously at 60 μM m^{-2} s^{-1} on average with a warm-white fluorescent tube (Mexico General Electric Co., Mexico) positioned horizontally along the side of the flasks. Ambient temperature was controlled at 23 ± 2 °C and the pH was 7.8. For cell synchronization each of the three 2-L flasks with culture were reinoculated every 3 days with 500 mL of cell suspension and 1500 mL of f/2 medium and this cycle was repeated twice prior to use with 12h:12h (light:dark). This was done in order to achieve synchronous growth and cell division of the microalgal cells. For experimental runs the doubly reinoculated suspension of microalgae was allowed to reach the fourth day and was used to prepare 9 different 1-L Erlenmeyer flasks (Kimble Glass Inc., USA) with 200 mL inoculum and 700 mL of f/2 medium for experimental runs. The experimental runs were repeated three times. Remaining inoculums were resynchronized as indicated above.

Photobioreactor System Using a 1-L Erlenmeyer flask a laboratory built photobioreactor system was proposed and designed using red and white LEDs for illumination (Fig. 1).

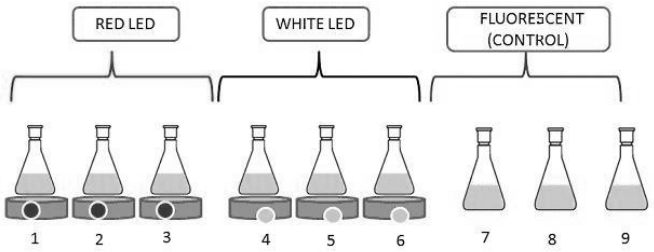

Fig. 1. Diagram of the proposed experimental system for production of microalgae with monochromatic light showing the 3 red LEDs (numbered 1,2 and 3) and 3 white LEDs (numbered 4, 5 and 6). The light generated from single LEDs applied to the base of an Erlenmeyer flask allows for the generation of light intensity of 60 μm mol·$m^{-2}s^{-1}$ equal to that of flasks 7, 8 and 9 (control group) using light from a white fluorescent tube (General Electric Co., Mexico) positioned horizontally to the side (not shown in diagram).

The system built consisted of 3 flasks for applying red LEDs and three for white LEDs and 3 control group flasks illuminated with the normal fluorescent lighting typically used to grow *I. galbana* in the laboratory. The red LEDs were GaAlAs-based with a maximum of wavelength 680 nm and half the bandwidth of 20 nm while the white LEDS used were GaN-based LEDs with a peak at about 465 nm (Steren, Mexico). Since the intensity of light decreases inversely by the square of the distance the LEDs were mounted directly to the base of the Erlenmeyer. Each LED illuminated flask base consisted of 4 components as shown in Fig. 2A: (1) LED, (2) Parabolic mirror, (3) lens and (4) a circular base made of PVC. An assembled 1-L photobioreactor is shown in Fig. 2B (right) covered with aluminum to exclude external light sources.

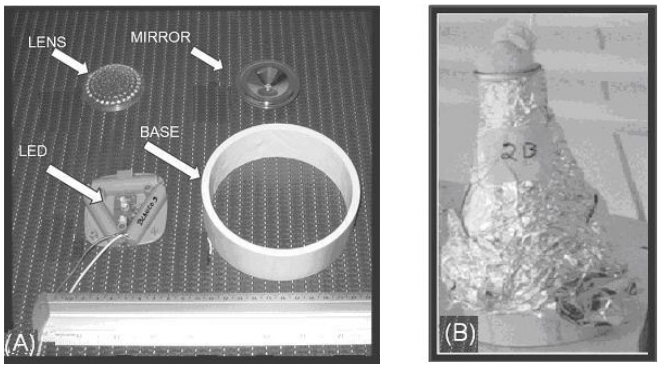

Fig. 2. Photographic detail of the elements of the experimental system for the production of microalgae with LEDs that shows in the photo on the left: (A) LED, lens, parabolic mirror and a circular-ring that provides the base support for an Erlenmeyer flask; to the right: (B) Is a flask in its base protected from external light with aluminum foil.

This scheme allowed for an illumination intensity equal to that of fluorescent lighting of 60 μM m^{-2} s^{-1} as measured at the middle of the suspension culture (around the 300 mL level). LEDs fail to generate any significant heating and thus LEDs can be placed very close to bottom of flasks without any heat stress to the microalgae suspension, LEDs do not generate heat and the temperature was measured to be 23 ± 2 °C. A warm-white fluorescent tube (Mexico General Electric Co., Mexico) was positioned horizontally along the side of the three control group flasks and the distance of the flasks from the fluorescent lighting source was adjusted to match that of the LEDS light intensity of 60 μM m^{-2} s^{-1}. The distance from the light source from the fluorescent (control) which was provided horizontally was adjusted so the horizontal lights provided were of the same intensity as the light from the LEDS from the bottom of the flask at mid level 200 mL. It is important to note that although the control light was provided horizontally and the experimental light was provided from the bottom of the flask, it is assumed that on average both microalgae cultures received similar light intensity doses due to fact that all flasks were air bubbled to provide mixing. The light intensity was measured using the 84022 SPER Scientific intensity meter (SPER Scientific Ltd., Scottsdale, AZ, USA) in lux and converted to μM m^{-2} s^{-1}.

In figure 3 is the actual system with LEDs assembled without flasks (left photograph)**,** while on the right is the actual system assembled with flasks in place.

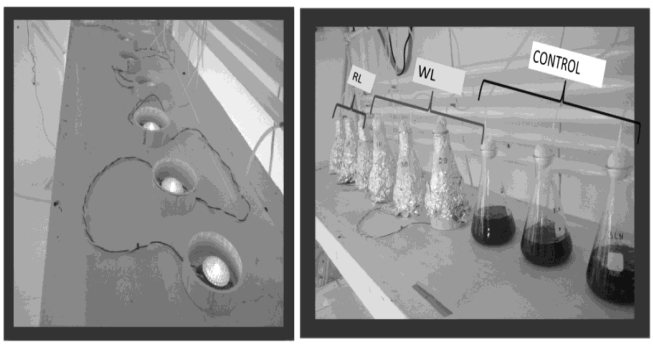

Fig. 3. Photographic detail of the elements of the experimental system shows : (A) the system with LEDs inside the base, and (B) the system with the 1-L flasks in light protected bases with external Aluminum foil (RL = red LED, WL = white LED, FL = Fluorescent light, *i.e.* the control group). The three flasks on the right are the control group and are illuminated on the side with a fluorescent light bulb.

Cell density At time zero and after each 5 days of light treatment the microalgae cell densities and cell size distribution were measured using a Coulter Counter

(Multisizer II; Beckman Instruments, Inc., USA) following the procedure described by Sheldon and Parsons (27). The Coulter counter not only provides total numbers but categorizes numbers of cells according to size. A 1:20 dilution was prepared by removing 1 mL of cell suspension per flask and adding of 19 mL of saline containing 10% buffered formol. The data of the Coulter Counter was converted by AccuComp® software and exported to a spreadsheet to calculate cell densities. Count numbers were multiplied by 20 (dilution factor). Samples were measured in triplicate. Size distributions due to light treatments were not obtained from this data since it has been documented that size numbers can be inaccurate with Coulter Counter when cells are nonspherical (28, 29, 30) or due to shrinkage caused by formaldehyde (31) to prevent cell growth.

Productivity (P) To obtain Productivity (P) in units of mg $L^{-1}d^{-1}$, the total dry weight of lyophilized microalgal cells was calculated by dividing its harvested dry biomass by culture volume (L) concentration and by the 5 days of light treatment.

Determining specific growth rate (μe) The specific growth rate of microalgae was calculated using the equation:

$$\mu_e = \frac{ln\left(\frac{N2}{N1}\right)}{t2 - t1} \qquad (1)$$

where μ_e = specific growth rate (d^{-1}), ***N1 and N2*** are the number of cells·mL^{-1} at the time *t1* and *t2*, respectively.

Duplication time The time for the number of cells (cells·mL^{-1}) of microalgae to double in number was calculated using the equation:

$$tg = \frac{l\eta 2}{\mu_e} \qquad (2)$$

where *tg* (days) is the time for cell duplication and μe is the specific growth rate, and *ln*2 is the natural logarithm of 2 (approx 0.693). This equation is derived from equation 1 by setting *N*2 = 2*N*1 and *tg* = *t*2-*t*1.

Microscopic measurement of cell size Live cell sizes after light treatment were measured with a Mytek USB 2.0 Digital Microscope Camera coupled to a microscope. A total of 9 cells were measured per each light treatment.

Protein quantification Protein content after the 5 days of light treatment was carried for all samples out based on the procedure of Lowry, et al. (32). The principle of this technique is based on the oxidation of the peptide bonds of the Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid), after treatment with an alkaline solution of copper, which results in a blue color with intensity dependent on peptide content. After lyophilization dry weight samples weighing 5 mg were placed in test tubes, then 5 mL of 0.1N NaOH was added to hydrolyze the proteins and then placed in thermal bath brand Techne TE-8J at 100 °C for 1 hour. The samples were allowed to cool to room temperature, and then the mixture was centrifuged at 3,000 rpm for 20 min at 10 °C. The extract was separated by Pasteur pipette. Two solutions were prepared: (1) Na_2CO_3 to 2% (w/v) in 0.1N NaOH (solution A) and (2) 0.5% $CuSO_4$ in 1% K-tartrate and stirred (solution B). An aliquot of sample (alkaline extract) plus 0.1 N NaOH to complete 1 mL was added in a test tube, and 5 mL of solution A and B in a 50:1 ratio was added, vortexed and was let to stand for 15 minutes. Later the mixture was added to 500 μL of Folin:H_2O in a ratio of 1:1. The mixture was vortexed and allowed to stand in the dark for 40 minutes. The calibration curve was prepared from a standard solution of bovine albumin (BSA) with a concentration range of 0 - 150 μg/mL (0, 30, 60, 90, 120 and 150 μg/mL). Absorbance of the extract was measured at 750 ηm in a Thermo Spectronic Genesys 20 spectrophotometer.

Carbohydrate quantification The carbohydrate content was determined using the method of Dubois et al. (33). The basis of this method is based on hydrolyzing glycosidic bonds forming polysaccharides and disaccharides to be converted to monosaccharides. The complexes vary in color from yellow to orange, depending on the amount of carbohydrates present in the sample. Lyophilized samples of 5 mg were weighed and placed in test tubes, then 1 mL of sulfuric acid (H_2SO_4) 1.0 N and the mixture was placed in an ultrasonic cleaner Branson 200 for 5 min. Then another 4 mL of sulfuric acid (H_2SO_4) 1.0 N was added to the mixture and the test tubes were sealed with aluminum foil along with a screw cap. These test tubes were placed in a thermal bath brand Techne TE-8J at 100 °C for 1 hour. The test tubes with mixture were

removed and allowed to cool to room temperature. Centrifuged at 3,000 rpm for 15 min at 10 °C and then separated the acid extract with a Pasteur pipette. An aliquot of sample (acid extract) plus 1.0 N H_2SO_4 to complete 1 mL was added into a test tube, and 1 mL of 5% phenol was added, mixed with a vortex and allowed to stand for 40 minutes. Then 5 mL of concentrated sulfuric acid (H_2SO_4) was slowly added in a fume hood, mixed with a vortex and cooled to room temperature. Absorbance was measured at 485 ηm in a spectrophotometer Thermo Spectronic Genesys 20 mark. The results were compared against a calibration absorbance curve prepared with 0, 24, 48, 72, 96, and 120 µg/mL, from a standard glucose solution of 120 µg/mL.

Lipid quantification Lipids were extracted according to Bligh and Dyer (34) adapted to micro determinations. Around 5 to 20 mg of freeze-dried sample was placed in individual test tubes, then 3 mL of chloroform/methanol/water (1:2:0.5) and 5 µL of antioxidant BHT (1 mg mL^{-1}) were added. The mixture was placed in an ultrasonic cleaner Branson 200, then samples were incubated for 24 h at 4 °C protected from light. Afterwards 2 mL of chloroform and 3 mL of water were added, mixed with a vortex and centrifuged at 3,000 rpm for 10 min at 10 °C. The bottom fraction (chloroform) was removed and placed in a test tube. An aliquot of sample (chloroform extract) was placed in a different tube and dried with N_2. Then 2 mL of concentrated sulfuric acid (H_2SO_4) was added and the test tubes were sealed with aluminum foil along with a screw cap. This mixture was burned at a temperature of 200 °C for 15 minutes according to Marsh and Weinstein (35). Then the fraction was allowed to cool to room temperature and placed in test tubes in an ice bath with 3 mL of distilled water and mixed with a vortex until the entire sample was homogeneous and no residual organic matter was visible. Burned lipids have brown color directly related with quantity. For the calibration curve a known quantity of lipids of 0, 30, 60, 90, 120, 150 and 180 µg was used from microalgae. Absorbance was measured at 375 ηm in a spectrophotometer Thermo Spectronic Genesys 20 mark.

Statistics Data was analyzed using Statistica 6 (Statsoft, Tulsa, OK). The Shapiro-Wilk test was carried out and was found that all data had a normal error of distribution. Subsequently, the Bartlett´s box test was run to insure sample variance was equal across samples or homogenous. Afterwards, analysis of variance (ANOVA) was run in conjunction with Tukey's test ($p < 0.05$) method for unplanned comparisons of means. For all measurements the number of samples consisted of three replicates per light treatment and was repeated three times (3x3, n=9).

RESULTS

Cell Density The cell density did not increase significantly in red or white LEDs after 5 days of treatment ($p > 0.05$), however the cell density of the control group with fluorescent lighting did increase significantly based on Tukey**'s** test ($p < 0.05$) after 5 days from 2.93 x 10^6 to 7.75 x 10^6 cells/mL (Table 1) a distinct difference when compared to red and white LEDs.

Table 1. Cellular density of *Isochrysis* aff. *galbana* (T-ISO) at time 0 and after 5 days of light treatment with white LEDS (WL), red LEDs (RL) and fluorescent lights (control) at 60 µm mol $m^{-2}s^{-1}$ (n=9).

	REGIME			RESPONSE		
Time	Light:Dark Period (hours)			Mean Cell Density (cells mL^{-1})		
days	WL	RL	FL	WL	RL	FL
0	12h:12h	12h:12h	12h:12h	2.66 ± 0.16x10^6	2.56 ±0.14x10^6	2.93 ± 0.19 x 10^6
5	12h:12h	12h:12h	12h:12h	2.94 ± 0.20x10^6	2.68 ±0.11x10^6	7.75 ± 1.01 x 10^6

Productivity and Specific growth rates Comparison of Productivity ($mg{\cdot}L^{-1}day^{-1}$) values of microalgae indicate that productivity was worse for red and white LEDS ~4 X compared to the control or fluorescent lighting ($p < 0.05$; Table 2). This is also reflected in lower Specific growth rates (μ_e) for white LEDs (4.8 X smaller) and red LEDs (4.2 X smaller) compared to the fluorescent lighting control group.

Growth or Duplication time The best or lowest mean duplication time (tg) was 3.7 days with fluorescent light control group and was significantly different from both LED light treatments ($p < 0.05$, Table 3).

The 3.7 day duplication time measured in *I. galbana* suggests at 5 days of light treatment the microalga had reached its maximum population growth with fluorescent lighting and had entered the saturation growth phase with delayed cell growth. We assume this because typical values of cell doubling time replication (tg) in the exponential phase for *I.* aff. *galbana* (T-ISO) is less than a day, for example approximately 9.5 h in other studies (36).

The values of cell replication (tg) for white and red LEDs were very high, 38.4 and 18.7 days, respectively, indicating that with these light sources the microalgae are not growing and suggest that microalgae are in stasis or a photorespiration phase, probably waiting for better light conditions for reproduction. A simple ratio comparison of duplication times indicates that with both white and red LEDs duplication was 10.4 X and 5 X longer, respectively, than with fluorescent lighting (control).

Table 2. *Isochrysis* aff. *galbana* (T-ISO) Productivity, P; Specific growth rate growth, μe (d^{-1}); and Time in days for cellular duplication, *tg* after 5 days of light treatment with white and red LEDs and fluorescent light at 60 μm mol $m^{-2}s^{-1}$(n =9).

	LIGHT SOURCE								
	WHITE LED			RED LED			FLUORESCENT		
Parameter	P	μ_e	tg	P	μ_e	tg	P	μ_e	tg
(units)	mg $L^{-1}d^{-1}$	(d^{-1})	(d)	mg $L^{-1}d^{-1}$	(d^{-1})	(d)	mg $L^{-1}d^{-1}$	(d^{-1})	(d)
MEAN	8.43	0.039	38.4	8.12	0.045	18.7	32.36	0.189	3.7
± STD DEV	2.98	0.040	3.4	0.32	0.023	9.3	4.72	0.024	0.5

Cell size obtained by microscopic photographs Digital images were taken (Fig. 4) after the light treatments (day 5) and showed that the size of the microalgae *I.* aff. *galbana* (T-ISO) is larger with fluorescent light (mean value of 49 μm^2), followed in size with white LED (mean value of 36.5 μm^2, $p < 0.05$), and smallest with red LEDs (mean value of 22.6 μm^2, $p < 0.05$), which corresponds to sizes that are 1.3 X and 2.2 X smaller, respectively, compared to fluorescent light or control group (n=9).

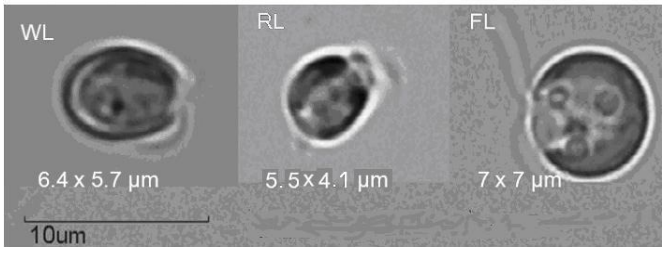

Fig. 4. Microscopic photographs of *Isochrysis* aff. *galbana* (T-ISO) showing typical differences in sizes with white LED (WL) , red LED (RL) and fluorescent light (FL = control group) after 5 days of light treatment in the experimental system at 1000X magnification (n =9).

Biochemical content Mean values of protein, carbohydrates, and lipids of *Isochrysis* aff. *galbana* at the end of 5 days of culture were very similar with red and white LED and fluorescent light (Table 3).

Table 3. Protein, carbohydrate and lipid biochemical composition of *Isochrysis* aff. *galbana* (T-ISO) at the end of 5 days of culture with red and white LEDs and fluorescent light (n =9).

	BIOCHEMICAL CONTENT		
LIGHT SOURCE	PROTEINS Mean ± STD (mg/g)	CARBOHYDRATES Mean ± STD (mg/g)	LIPIDS Mean ± STD (mg/g)
WHITE LED	301 ± 28	75 ± 19	388 ± 68
RED LED	322 ± 57	87 ± 37	356 ± 77
*FL	276 ± 47	80 ± 10	378 18

*FL= fluorescent light (control)

DISCUSSION

According to the present findings the cell density growth rate did not significantly differ from zero after 5 days of light treatment in red and white LEDs. This result differs from the limited studies with LEDs with other microalgae, dinoflagellates or cyanobacteria where growth is observed irrespective of whether treated with either red or blue LEDs (17,18,19,20). However, this is significant for as far as the authors are aware this is the first study that *Isochrysis* aff. *galbana* (T-ISO) has been cultivated, or attempted to be cultivated with LED-derived light sources, herein monochromatic red and multi-chromatic white LEDs. Also, all previous reported studies with LEDs and microalgae have used green microalgae, while *I.* aff. *galbana* (T-ISO) is a golden-brown microalga.

The result is surprising since it is well known that in *I.* aff. *galbana* the main pigments are chlorophyll *a,* c1, c2 and fucoxanthin (37). Chlorophyll *a* is considered generally to be sufficient to drive photosynthesis and it strongly absorbs in the red and blue region. However, the importance of antenna

pigments photon capture is well recognized at least in cyanophytes. The transfer of energy trapped by phycobilisomes (PBS) to the chlorophyll a of photosystem I (PS I) or photosystem II (PS II) is regulated either at the transfer point from the PBS to the two photosystems or at a transfer point between the Chlorophyll a of PS II and PS I (38). It is important to note, that white light LEDs used in this study are actually mainly a high power blue emitter LED with some phosphor in conjunction, the current technical solution and as such is not true white light. One possibility is that there was not enough energy at the specific wavelength to activate antenna pigments like fucoxanthin.

It is hypothesized that although cell sizes were very different, the percentages or ratio of biochemical content was maintained irrespective of biomass loss. This may be a fitness strategy that the microalgae have maintained during inadequate light intensity or wavelength in order to survive while waiting for better light resources for reproduction.

Herein, we also report that red and white LED-derived light leads to a reduction in cell size compared with fluorescent lighting in this non-green microalga, *Isochrysis* aff. *galbana* (T-ISO). Studies of microalgal cell size changes induced by light quality are few. However, we found at least one study in the literature that reports a similar effect, but with a green freshwater microalga. Lee and Palsson (18) reported that with *Chlorella vulgaris* a narrow-spectrum monochromatic red light reduced average cell volume from 60 to 30 μm^3 $cell^{-1}$, and also found by switching light sources to full spectrum that the two dissimilar cell population states were interchangeable.

Another possibility of the size reduction observed with red and white LEDs is that the light fluence level applied is below the light intensity compensation point (38) and the microalga is smaller simply because its respiration rate is higher than its photosynthetic rate and it utilized its energy stored. We suggest that this indeed may be a possibility, but not because the fluence rate is not high enough, 60 $\mu M\ m^{-2}\ s^{-1}$ is more than sufficient, but possibly because *Isochrysis* aff. *galbana* (T-ISO) needs another specific wavelength to grow.

In summary, the data in this study suggest that *Isochrysis* aff. *galbana* (T-ISO) needs a wavelength possibly a green wavelength to grow and divide other than the red and white LEDs provided for in this study for the following reasons: (i) it is generally accepted that the minimum photosynthesis level for unicellular photoautotrophic growth, although at low growth rates, is at 2 $\mu M\ m^{-2}\ s^{-1}$ (40) our fluence rate was well above this at 60 $\mu M\ m^{-2}\ s^{-1}$, (ii) Lee and Palsson (18) observed growth in *Chlorella* (a green microalgae) with narrow-bandwidth red light with LEDS, but their microalgae was green with obviously different pigments or ratio of pigments, (iii) *Isochrysis* aff. *galbana* (T-ISO) is a yellow-brown microalgae haptophyte possibly indicating it has pigments like fucoxanthin as part of the main auxiliary pigments (38) which absorbs between 480 and 530 nm (41) mostly corresponding to green light, and (iv) many earlier landmark studies with photosynthesis and with microalgae have shown that a combination of different wavelengths affects photosynthesis rates (39) and this is due to accessory pigments (42); however, most of these studies were conducted with green or red microalgae.

CONCLUSION

Hence, the results suggest that the non-growth of *Isochrysis* aff. *galbana,* a golden-brown colored haptophyte, with red or white (mainly blue) LEDS is because the microalgae needs another wavelength to activate auxiliary pigments. This conclusion is supported by two facts: (1) the microalgae did not divide with LEDs (numbers were essentially the same before and after light treatment with LEDs) and (2) the size of the microalgae with LEDs was significantly smaller compared to fluorescent (control) light treatments, suggesting that with LEDs the microalgae was only in a respiration phase and was using its energy stores. It is also important to note that this microalga is a different pigmented microalga than that was used in the earlier pioneering studies of photosynthesis which used green and red microalgae, a possibly reason why this response has not been previously reported. Moreover, it is still unclear whether a single wavelength or combinations of wavelengths are necessary to influence photosynthesis or photomorphological responses in this microalga studied, *i.e. Isochrysis* aff. *galbana*. Further light studies will be required to confirm and disentangle the light processes in this microalga.

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