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Enhancement of CO_2 biofixation and lipid production by Chlorella vulgaris using coloured polypropylene film.

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Abstract

The microalgae *Chlorella vulgaris* (*Cv*) was cultivated with light at different wavelengths (λ_{max}) and irradiation intensities (*I*) by applying coloured tape (CT) as a simple, inexpensive solar light filter. *Cv* was cultivated in a standard medium using blue (CT_B), green (CT_G), red (CT_R), yellow (CT_Y), and white (CT_W) coloured tape to filter the light, as well the unfiltered light (U). The CT-filtered light spectrum was characterized in terms of λ_{max} and *I*, and the influence of these parameters on algal growth (specific growth rate, μ), nutrient removal efficiency (% RE of total nitrogen, TN, and phosphorus, TP), CO_2 fixation rate (R_C) and lipid productivity (P_{lipid}) evaluated. Growth and nutrient removal parameters were normalised against *I* for comparison.

For the un-normalised data the order of the growth and lipid productivity parameters was CT_W> unfiltered light (U) ≈ CT_Y > CT_R > CT_B. The highest biomass concentration X_{max} of 2.26 g L^{-1} was measured for CT_W with corresponding μ, TN and TP RE, R_C and P_{lipid} values of 0.95 d^{-1}, 92% and 100%, 0.67 g L^{-1} d^{-1} and 83.6 mg L^{-1} d^{-1} respectively. For the normalised P_{lipids} parameters, however, the order of growth impacts CT_W > CT_Y > CT_R > CT_G > U > CT_B. The normalised algal growth and P_{lipids} parameters for U were significantly lower than in CT_W of 33-50% and 75% respectively. The corresponding non-normalised parameter values for CT_B were significantly lower at 0.45 d^{-1}, 0.18 g L^{-1}, 15% and 37%, 0.03 g L^{-1} d^{-1} and 1.2 mg L^{-1} d^{-1}. Results suggest a significant efficiency impact of both light intensity and wavelength, with up to a 50% increase in growth and an associated improvement in nutrient removal efficiency from optimising these two parameters.

Keywords: *Chlorella vulgaris*; *Solar light*; *wavelength, colour tap, Specific growth rate.*

1 Introduction

Light plays a key role in microalgae cultivation, with light intensity (*I* in μE m^{-2} s^{-1}) and quality, most readily defined by the wavelength λ_{max} in nm, both known to significantly influence the cell growth rate [1-3]. These parameters should therefore both be considered when selecting the light source for microalgae cultivation.

Generally, reduced light intensity commensurately reduces algal growth whereas excessive lighting can lead to photo-oxidative damage of the cells [4-7]. There is further an impact on efficiency: low light intensities can lead to photosynthesis efficiencies (PE) of up to 80% of the theoretical maximum of 0.125 mol CO_2 fixed per mol photons absorbed [4-7]. However, light utilisation is itself dependent on algal cell concentration. For a high cell-density culture, the light energy is predominantly absorbed close to the photobioreactor (PBR) walls, since it cannot penetrate deep into the bioreactor due to dissipation by the cells. The algal cells are thus exposed to *I* values beyond those which can be completely utilised for biochemical energy through photosynthesis. This oversaturation leads to light energy lost through heat dissipation [8], resulting in a reduced PE compared with that obtained at lower *I*.

Table 1: Solar light filter and LED reported in the literature for *Chlorella vulgaris* (*Cv*)

Light source	*LF or LED char.*	*Wavelength λ, nm*	*Cult. Med.*	*System Confi.*	*TP_{in}, mg L^{-1}*	*TN_{in}, mg L^{-1}*	*Inlet CO_2 C_{cg}, % v/v*	*Light int, μE*	*X_{max}, g L^{-1}*	*Cd, Cells mL^{-1}*	*μ, d^{-1}*	*RE TP, %*	*RE TN, %*	*CO_2 fixn. rate RC, g L^{-1} d^{-1}*	*Lipids pr. P_{lipids} mg L^{-1} d^{-1}*	*HRT, d^{-1}*	*Ref.*
Cell p.	Thi. 3mm	λB, 460	MW	M.B	4	36	0.03[a]	50	0.47	nr	nr	57	72	0.05[1]	0.008[2]	nr	[9]
		λR, 620							0.36	nr	nr	45	42	0.04[1]	0.007[2]		
	Btc Pr	C,620,540,430							0.27	nr	nr	37	38	0.03[1]	0.003[2]		
	LF	λG, 540							0.12	nr	nr	35	16	0.01[1]	0.002[2]		
Lumi AS	color dyes	λG	3N-BBM +V	nr	31	4.1	nr	200-250	nr	43×10^6	0.1	nr	nr	0.05	nr	nr	[10]
		λO, 585-620							nr	42×10^6	0.13	nr	nr	0.05	nr		
	Btc Pr	λR, 600-700							nr	36×10^6	0.07	nr	nr	0.02	nr		
	LF	λv, 400-450							nr	43×10^6	0.11	nr	nr	0.05	nr		
Lumi AS	color dyes	λB	3N-BBM +V	BCPBR	31	4.1	0.03[a]	250	nr	41×10^6	0.5	nr	nr	0.21	nr	90-45	[11]
		λG							nr	43×10^6	0.51	nr	nr	0.25	nr		
	Btc Pr	λY							nr	41×10^6	0.36	nr	nr	0.22	nr		
	LF	λO							nr	40×10^6	0.37	nr	nr	0.21	nr		
		λR, 600-700							1.5	45×10^6	0.42	nr	nr	0.24	nr		
LED	W= 26 mm	λR, 620-630	SDS	EF	5.2	43	nr	1000	0.28	nr	nr	41	55	0.0484	nr	42	[12]
		λY, 590-600							nr	nr	nr	nr	nr	nr	nr		
	L= 600 mm	λB, 460-470							nr	nr	nr	nr	nr	nr	nr		
		λG, 525-550							nr	nr	nr	nr	nr	nr	nr		
	Btc Pr	λW, 380-760							nr	nr	nr	nr	nr	nr	nr		
LED	Btc Pr	λB, 460	Z8	nr	nr	nr	0.03[a]	100	0.81	nr	nr	nr	nr	0.20	0.013[2]	nr	[13]
		λG, 535							0.63					0.16	0.009[2]		
		λY, 585							1.59					0.40	0.031[2]		
		λR, 620							1.26					0.31	0.021[2]		
		λW, 400-700							1.33					0.33	0.026[2]		
LED	Btc Pr	λB, 430-460	MJ	EF	nr	nr	0.03[a]	100	0.78	17.5×10^6	nr	nr	nr	0.27**	nr	nr	[14]
		λR, 620-665							0.84	25×10^6	nr	nr	nr	0.29**	nr		
		λW, 400-700							0.8	20×10^6	nr	nr	nr	0.28**	nr		

Cell p.= cellophane papers; LF= light filter; char.=characterization; cult.med.= cultivation medium; thic.=thickness; pr.=production; MW= municipal wastewater; M.B= media bottle; Btc P= batch process; LED= light emitting diode; λ= wavelength number, B=blue, R= Red, C= control, G= green, O=Orange, V= violate, Y= Yellow, W= white, SDS= synthetic domestic sewage; W= width; L= length; EF= Erlenmeyer flask; Z8= standard medium for green algae; MJ= standard cultivation medium; 3N-BBM+V= bold basel medium; Lumi AS= Luminescent acrylic sheets contains dyes; BCPBR= bubble column photobioreactor; HRT = hydraulic residence time, [a]Atmospheric level, [1]= calculated from *Rc* = CO_2 fixation rate which estimated from Chisti ratio: $CO_{0.48}H_{1.831}N_{0.11}P_{0.01}$; $Rc = 1.88 \times P_x$, P_x = biomass productivity which estimated from $\Delta X/\Delta t$,[2]= calculated from Eqs.1; Not reported= nr.

The above implies that optimization may be possible through manipulating the light conditions by providing an improved balance between light capture and the photochemical process [15-17], thereby enhancing the PE. This can be achieved through minimizing the light absorption by shifting the wavelength of emitted light to the weakly absorbed green region through the use of a light filter (LF) or light emitting diode (LED), as demonstrated in recent studies on the most commonly studied *Chlorella vulgaris* (*Cv*) algal species (Table 1).

A key constraint on implementation of an optimised λ_{max} is the LF or LED capital cost. However, potentially low-cost materials are available that may suit this duty. The coloured tape (CT) solar light filter material is a biaxial oriented (i.e. extruded in two directions) polypropylene (BOPP) film. Applications of the material are diverse, and include food packing protective coating, pressure sensitive tape, label printing, metallizing and decorative products [18-20]; its use has been extended to many different applications by coating technology with solvent based acrylic adhesive [23] which makes it highly transparent with excellent optical properties [23]. Since the material is produced in different colours, it can be used to filter light to select the appropriate wavelength.

The current study employs CTs to filter light to attain a specific λ_{max} for a given *I*. The impact of the changing light characteristics on algal growth, nutrient removal, CO_2 fixation and lipid productivity has then been evaluated.

2 Material and methods

2.1 Pre-culture preparation and analysis

The *Chlorella vulgaris* (*Cv)* algal strain (CCAP 211/11B, CS-42) was used as described previously [21, 22]. Experiments were conducted in 350 mL cylindrical glass columns (ID = 4 cm), each with a 250 mL working volume. The standard MLA (*Marine labs American society of microbiology-derived medium*) medium has been described elsewhere [22]. 250 ml batches of sterilized medium were inoculated by 1 vol% pre-cultured *Cv* with initial cell concentration of 0.3×10^6 cells mL^{-1}. The culture was continuously fed with a flow of 50 mL min^{-1} filtered air (0.03 % CO_2), adjusted by digital mass flow controllers (MC-100SCM, Cole-Parmer, USA). All experiments were conducted at a temperature of 22-25°C. Continuous illumination at a light intensity (*I*) of 250 µE m^{-2} s^{-1}, provided by adjusting the number of 8W LED lights, was measured by a light meter (LI-250A, LI-COR, US). A 5 mL sample was extracted daily for analysis, equating to a hydraulic and solids residence time of 50 days, and all runs lasted for 10 days.

Nutrient concentrations of the 0.45 µm-filtered liquid samples were determined colorimetrically using HACH test kits (DR/890 Colorimeter, HACH, USA) and the total organic carbon (TOC) concentration determined using a Shimadzu TOC analyser (TOC-VCPH, Shimadzu, Japan). The optical density was determined by UV-Vis spectrophotometry (Jasco *V-670*, JASCO Corporation, Japan) at 680 nm, and the reading converted to dry cell weight (DCW g/L) by calibration. The specific growth rate μ was then calculated from the initial and final biomass concentrations and the corresponding cultivation time. For all nutrient tests the control sample contained 6 mg L^{-1} TP and 28 mg L^{-1} TN, based on the typical medium MLA composition stipulated by the supplier. Microalgal cells were enumerated using a biological light microscope (ACHRO 40/0.65, Saxon, New Zealand). CO_2 capture (R_C) is given by given by $C\ P_X\ M_{CO2}/M_C$, where *C* is the dried cells % carbon content, measured by an element analyser (CHNS/O analyser, PerkinElmer, USA), P_X the biomass productivity, and M_{CO2} and M_C are the respective molar weights of CO_2 and carbon.

2.2 Lipids extraction and quantification

Algal cells were harvested by centrifugation at 5000 rpm for 15 min (C-28A, BOECO, Germany) and the supernatant decanted. The cell pellets were washed with distilled water and then freeze-dried at -50 °C for 15 hrs (Alpha 2-4 LDplus Laboratory Freeze Dryer, Christ, Germany). The total lipids were then extracted from microalgae biomass using a modified method of Blight and Dyer [23]. 50 mg of lyophilized algal biomass was placed in a 15 mL test tube and mixed with a 2:1 chloroform-methanol solvent. The mixture was ultrasonically treated at 0.4 W mL^{-1}, with a pulse of 55/5 and an amplitude of 90%, at 20°C for 10 minutes (Vibra cell, Sonic Materials, USA). The chloroform-methanol phase containing the extracted lipids was then separated and the solvent removed using a vacuum rotary evaporator (R-201, Rose Scientific Ltd, Canada) at 5 psi pressure and temperature of 70°C.

The lipids productivity (P_{lipid}) was calculated from:

$$P_{lipid} = \frac{X_{max} \times Y(\%)}{V \times C_p} \quad (1)$$

where X_{max} is the cumulative microalgae biomass production (g), Y the %lipids content, V the working volume (L) and C_p the cultivation period.

2.3 Coloured tape light filter (CT)

To evaluate the effect of light wavelength on the wastewater treatment and microalgae growth, coloured tape (CT) of different colours (red, blue, yellow, green and white) was used to filter the light. CT spectral characteristics, from spectra analysis software (Jasco *V-670*, JASCO Corporation, Japan), indicated them to produce illumination mainly in the visible range (Fig. 1, Table 2). This implies an associated reduction in energy (i.e. longer wavelengths) from that of the incident white light. The CTs were subsequently directly wrapped around the PBRs to select the appropriate irradiated light wavelength range (Fig. 2).

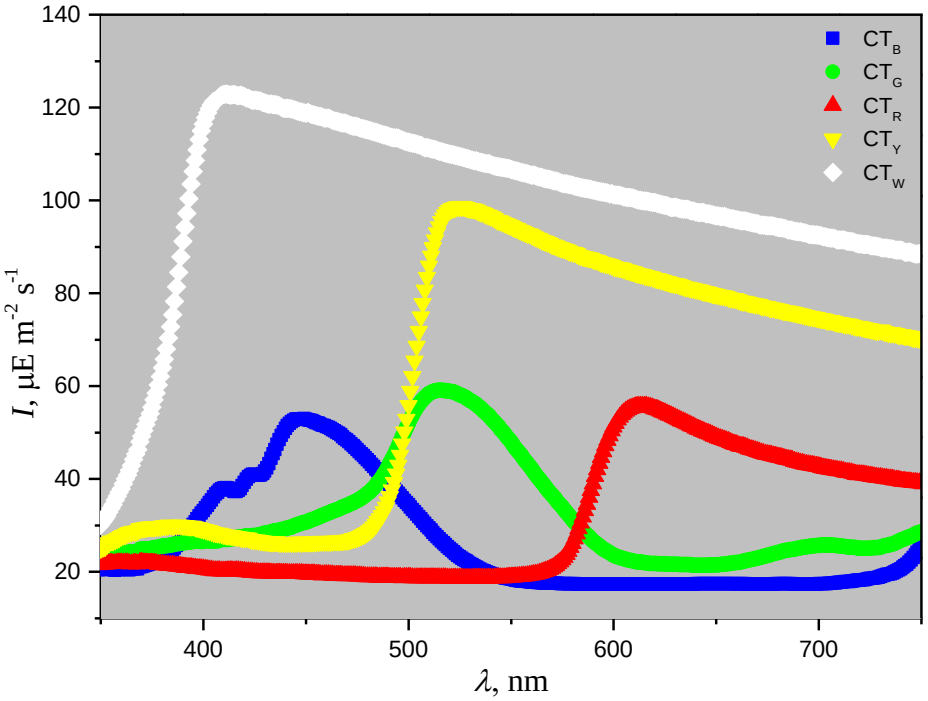

Figure 1: LQ variation at different LU (λ_{peak}) CT of various colours: white (CT_W), blue (CT_B), green (CT_G), yellow (CT_Y) and red (CT_R).

Table 2: CT film characterization, 48 mm width tape

Colored tape (CT_s)	*Wavelength range, nm*	*Peak wavelength* λ_{peak}, *nm*
White, CT_W	750-350	413
Blue, CT_B	549-345	451
Green, CT_G	600-400	518
Yellow, CT_Y	750-481	528
Red, CT_R	750-575	616

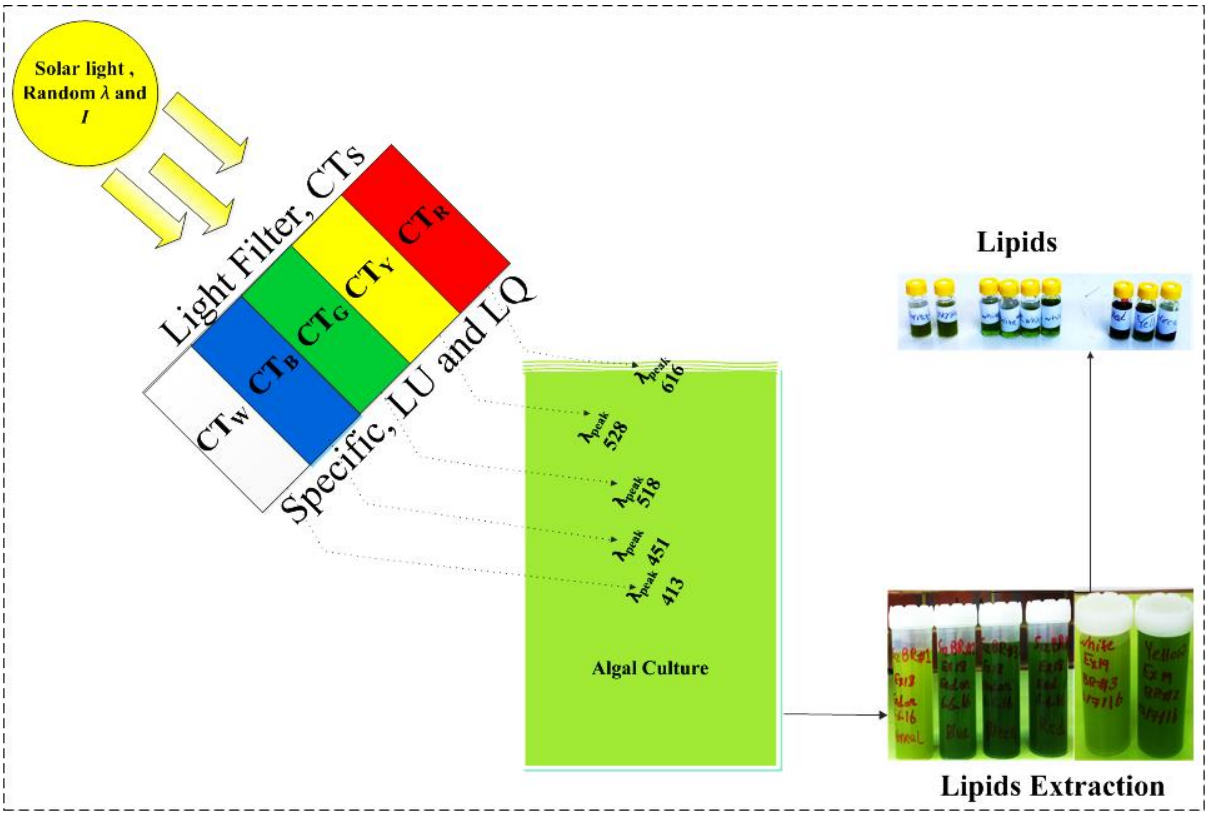

Figure 2: Biofuel production cycle, schematic

3 Results and discussion

3.1 Influence of I and λ_{max} on Cv growth

The biomass concentration (X), specific gowth rate (μ) and the *Cv* cell density (C_d) were evaluated at different wavelengths using the selected filtered light sources, namely CT_B, CT_G, CT_R, CT_Y and CT_W along with a control using the unfiltered light source (U) (Fig. 3). The highest X of 2.26 g L^{-1} was obtained with CT_W along with μ and Cd values of 0.95 d^{-1} and 54×10^6 cells mL^{-1} respectively. The corresponding values obtained with U were 1.14 g L^{-1}, 0.64 d^{-1} and 28.16 $\times10^6$ cells mL^{-1}. Against this, lower X, μ and C_d values of 0.18 g L^{-1}, 0.45 d^{-1} and 2.8 $\times10^6$ cells mL^{-1} were respectively recorded for CT_B. The values of 1.02 g L^{-1}, 0.61 and 25.9 cells mL^{-1} measured for CT_Y were comparable with those for U. The μ value obtained for CT_R and CT_G were comparable at 0.55 d^{-1} and 0.58 d^{-1} respectively, although slightly higher X and C_d values were measured for CT_R compared with CT_G (0.91 vs. 0.75 g L^{-1} and 25.6 vs. 22.25 cells m L^{-1} for CT_R and CT_G respectively).

CT_W provided a light wavelength range of 750-350 nm, with a peak of 413 nm, and reduced the control *I* (U) of 250 µE m^{-2} s^{-1} by about 50%. Non-photochemical quenching (NPQ) is known to arise if the rate of photo-inhibition exceeds the rate of repair, resulting in a large proportion of the captured light being dissipated at high *I* [24]. The lower *X* and μ values obtained for U suggest that growth is reduced at longer random wavelengths [10]. It has been suggested that the U spectrum supplied for microalgae cells does not necessary cover the absorption bands of microalgae pigments [27]. U may also contain the absorption bands of chlorophyll pigments of microalgae, or may comprise a combination of growth efficient and inefficient light spectra [25]. Recorded growth rates were higher than those of Kim et al [14], who reported *X* and C_d values of 0.78 g L^{-1} and 17.5× 10^6 cells mL^{-1} respectively using LED lighting providing a wavelength band of 400-460 nm and an *I* of 100 µE m^{-2} s^{-1}. A 78% reduction in incident *I* to approximately 55 µE $m^{-2}s^{-1}$ was recorded for CT_B, decreasing the level of photosynthesis active radiation (PAR) and thus photosynthesis activity accordingly [24]. At lower *I* up to 80% of the theoretical maximum photosynthesis efficiency (PE) can be achieved [4-7], although a reduced *I* value results in a proportionally lower biomass production. Maximum algal growth rates result from optimum combination of the appropriate average irradiance of cells with the higher photosynthetic efficiency [26].

Similar growth patterns were obtained for CT_Y and N_R despite a ~60% reduction in *I* for CT_Y, which has λ_{max} of 750-481 with a peak of 528 nm (Table 2). This wavelength range has been reported as being optimal for an X_{max} of 1.5 g L^{-1} based on an initial light intensity of 250 µE m^{-2} s^{-1} [11]. Reduced growth rates arose for CT_R and CT_G, with an almost 70% reduction in *I* with corresponding λ_{max} of 400-600 with peak of 518 nm, and 750-575 with a peak of 616 nm, respectively. The longer wavelengths/lower energies associated with CT_R have been reported to improve the algal growth in a PBR by sustaining light penetration through improving mixing within the reactor and increasing the depth of light penetration under higher light intensity [10, 11].

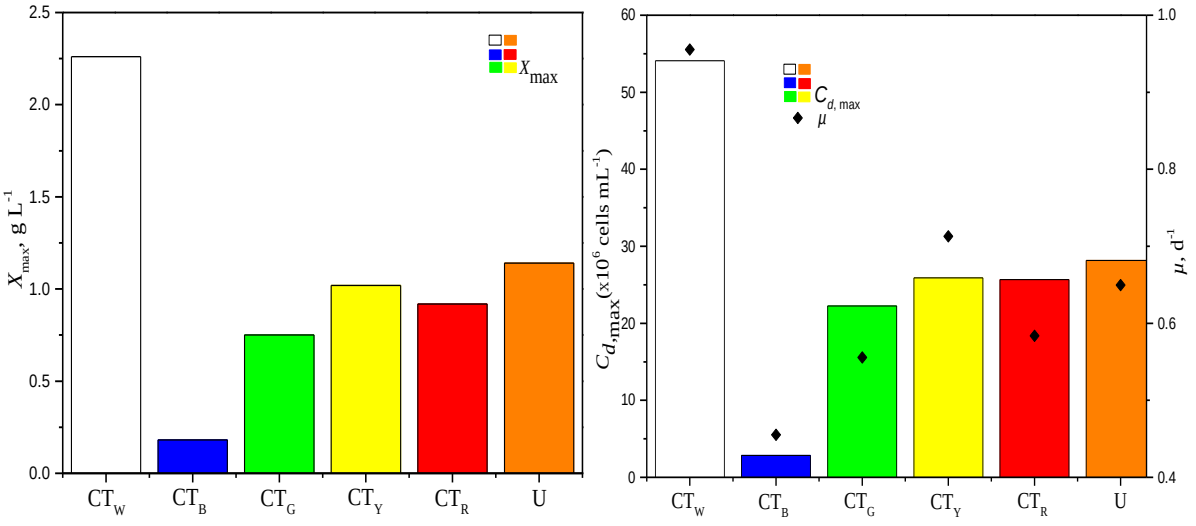

Figure 3: Cultivation of *Cv* in MLA under different LU and LQ: (a) *X* profiles, and (b) μ and C_d as function of λ_{max} and *I*.

3.2 Nutrients removal under different LQ and LU

The correlation of nitrogen and phosphorus removal efficiency (RE TN and RE TP) by *Cv* with wavelength (Fig. 4) indicates the expected maximum removal (92% RE for TN, 100% for TP) for CT_W in accordance with growth rate trends. Removal otherwise appeared to peak

for CT_Y (65% RE for TN, 72% for TP), and was significantly lower (15-18% for both nutrients) for CT_B. The results show there is a significant difference in the RE TN obtained in CT_W and N_R - about 1.27 fold higher for CT_W - attributable to the lower λ_{max} of the unfiltered light source. TP RE for CT_W and CT_N were similar at 92% and 90% respectively.

P removal is impacted more than N removal by pH via abiotic precipitation, although assimilation by algae remains the primary P removal mechanism [21]. The CT_W filtered spectrum contains a wide variety of wavelengths, peaking at 413 nm, that can significantly enhance *Cv* growth and so nutrient uptake. CT_W thus appears to offer an optimal light wavelength band for removing both TN and TP in terms of both λ_{max} and *I*.

Removals overall are significantly greater than those reported by Kang et al [9] for the same algal species using cellophane wrapping paper as light filter. These authors reported TN and TP REs of 41% and 55% for a 620-630 nm wavelength based on a 42-day cultivation time in a batch operation with an *I* of 50 µE m^{-2} s^{-1}, compared with 99-123 µE m^{-2} s^{-1} for a 413-528 nm wavelength irradiation at 50 days HRT in the current study (Table 1).

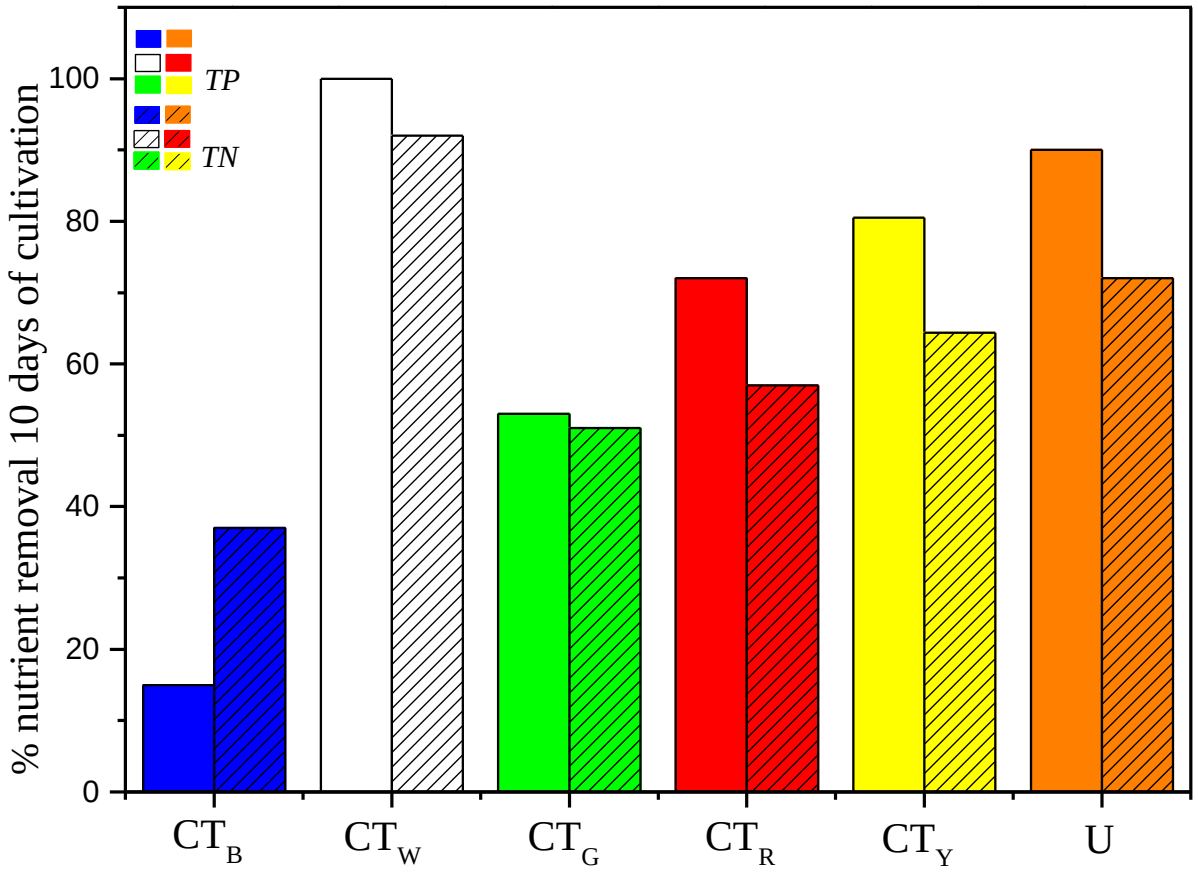

Figure 4: Specific growth rate and TP, TN removal efficiencies.

3.3 λ_{max} and I impacts on lipids production of Cv

Lipid productivity (P_{lipid}) and CO_2 fixation rate (R_C) under the different light conditions follow a similar pattern to that of growth and nutrient removal, with CT_W providing the greatest lipid production and CO_2 fixation and the trend otherwise peaking for CT_Y (Fig. 5). According to Ruysters [28] a λ of 400-500 nm assists in the regulation of gene transcription and activation of enzymes. In the biosynthesis process of lipid, the two key components of

acetyl-coA carboxylase (AAC) and nicotinamide adenine dinucleotide phosphate (NADPH) are produced by the concerted actions of ATP citrate lyase (ACL), malic enzyme (ME), and fatty acid synthase (FAS); these enzymes are effectively active only at wavelengths of 400-500 nm [29].

The results are comparable to those of Kim et al [14], based on a batch process using LED with wavelength ranges of 620-665 nm, 430-460 nm, and 400-700 nm, and an intensity of 100 µE m^{-2} s^{-1}. However, P_{lipid} reported in [13] for a batch LED process providing mean wavelengths of 460, 535, 585 and 620 nm using the same were significantly lower than in the current study (Table 1).

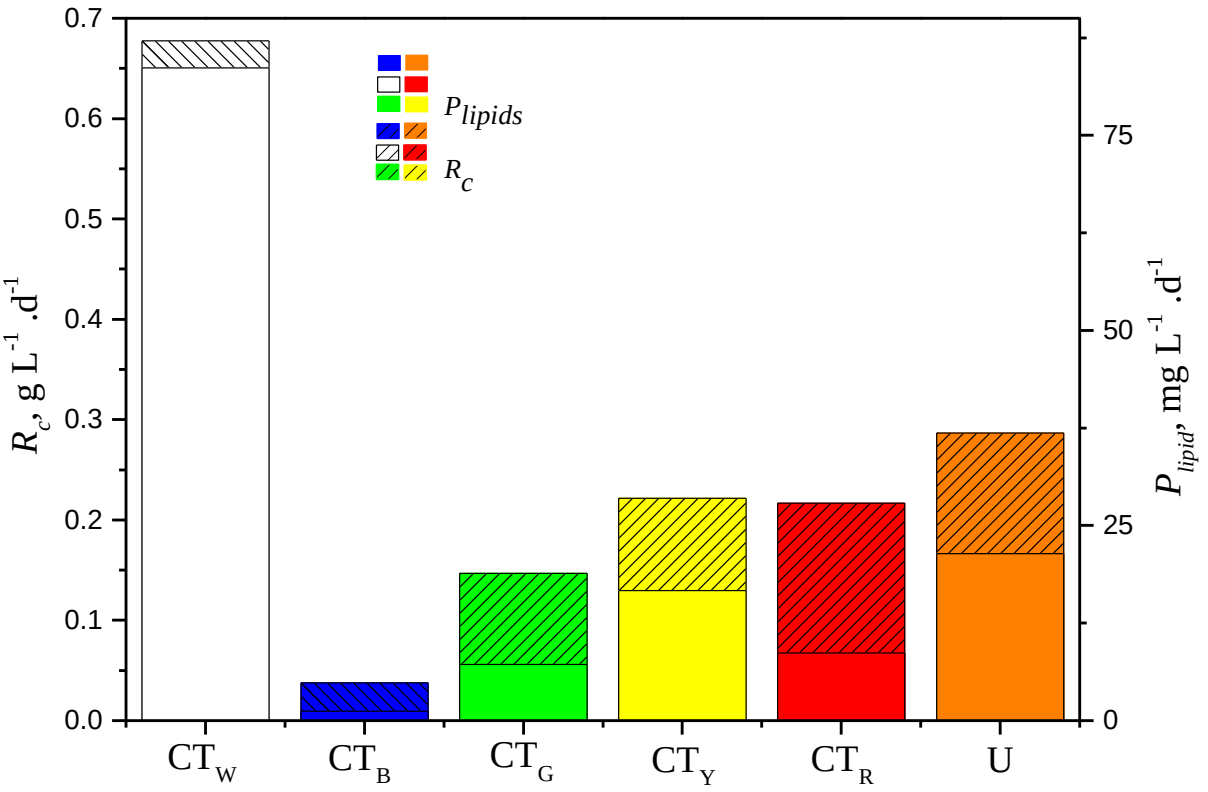

Figure 5: CO_2 fixation and biofuel production rate at different wavelength.

3.4 Growth and productivity normalisation

Normalisation of key growth and lipid productivity parameters against irradiation intensity indicates significant differences between the different filtered light sources (Fig. 6). Previous studies based on the same algal strain using an unfiltered light (U_P) source at light intensities of 100 and 50 µE m^{-2} s^{-1} compared with 250 µE m^{-2} s^{-1} in the current study and [30] suggests unfiltered light to be 33-50% less effective at promoting algal growth and 75% less effective at promoting lipid productivity than filtered CT_W in the current study. U_P indicated a similar trend of about 20-30% reduced influence in supporting algal growth compared with CT_W, although there was a slight enhancement in U_P of about 13-20% in algal growth compare with U as a results of lower light intensity used in U_P. The optimum irradiance intensity for maximum *Cv* growth was previously reported as being 100 µE m^{-2} s^{-1} [24, 30].

Normalisation of algal growth parameters against *I* yields similar values of the specific growth rate across all filtered light tests, whereas the normalised maximum algal biomass

concentration was 4-5 times lower for blue light (λ_{peak} = 451 nm) than for longer light wavelengths (λ_{peak} > 518 nm). Normalised lipid productivity was similarly 85% less for blue light than for the longer light wavelengths, and <5% of the value determined for filtered white light. Excessive irradiation (250 µE m^{-2} s^{-1}) appears to be detrimental to algal growth and lipid productivity, as is light in the blue range, although reducing the light intensity to below (250 µE m^{-2} s^{-1}) enhances the algal growth in U_P.

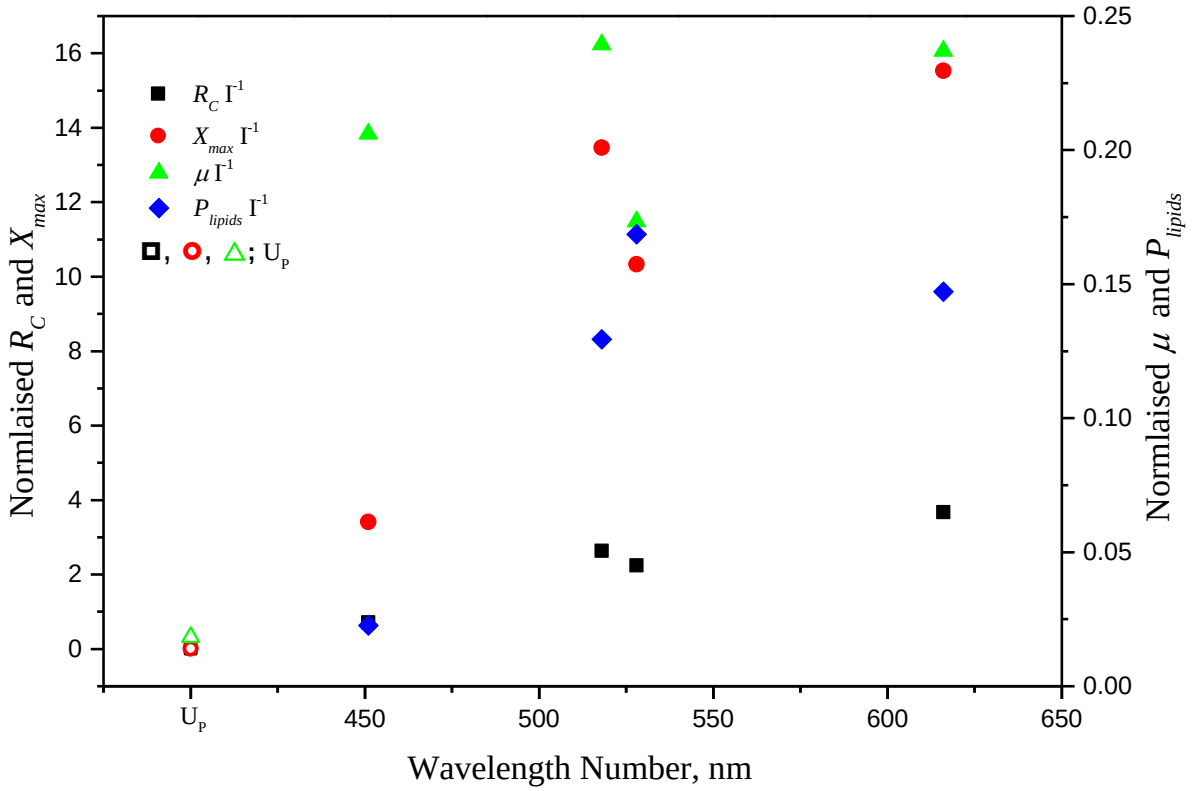

Figure 6: Normalised growth parameters and lipids productivity against light wavelength and [31] data (U_P).

4 Conclusions

The influence of light wavelength (λ_{max}) and irradiation intensity (I) on the specific growth rate μ, nutrient removal efficiency (RE of nitrogen N and phosphorus P), CO_2 fixation (R_C) and lipids production (P_{lipid}) of microalgae *Chlorella vulgaris* (*Cv*) has been investigated. Coloured tape (CT) based on the colours blue (CT_B), green (CT_G), red (CT_R), yellow (CT_Y), white (CT_W) was used as a low-cost means of adjusting the wavelength, and the outcomes compared to unfiltered light (U).

The results revealed that the use of CT to filter light reduced I which then generally benefitted the algal growth through suppressing photo-inhibition. The order the order of the growth and lipid productivity parameters was CT_W> U ≈ CT_Y > CT_R > CT_B. Filtered white light (CT_W) was found to enhance growth, giving X_{max}, R_C, μ and P_{lipids} values of 2.26 g L^{-1}, 0.67 g L^{-1} d^{-1}, 0.95 $d^{-1}$and 83.6 mg L^{-1} d^{-1} respectively compared with 1.14 g L^{-1}, 0.28 g L^{-1} d^{-1}, 21.3 mg L^{-1} d^{-1} for U. CT_W provided significantly faster growth than colour-filtered light (CT_Y, CT_R, CT_G and CT_B), in part due to the greater I. Similarly, CT_W was also more beneficial for N and P removal, at 92% and 100% respectively. Lowest growth-related parameter values were recorded for CT_B, with μ, X_{max}, R_C and P_{lipids} of 0.45 d^{-1}, 0.18 g L^{-1}, 0.03 g L^{-1} d^{-1} and 1.2 mg L^{-1} d^{-1} respectively. Results for CT_Y were otherwise comparable

with U, at 0.61 d^{-1}, 1.02 g L^{-1}, 0.22 g L^{-1} d^{-1} and 16.6 mg L^{-1} d^{-1}; slightly reduced growth for CT_R and CT_G was recorded.

Normalisation of algal growth parameters against *I* yielded similar values of μ across all filtered light tests, but indicated a change in the order of growth impacts overall to CT_W > CT_Y > CT_R > CT_G > U > CT_B: unfiltered light was reduced in efficacy compared with the un-normalised parameters. Blue light remained significantly less effective: the normalised maximum algal biomass concentration was 4-5 times lower for CT_B (λ_{peak} = 451 nm) than for longer light wavelengths (λ_{peak} > 518 nm). Normalised lipid productivity was similarly 85% less for blue light than for the longer light wavelengths, and <5% of the value determined for filtered white light. Excessive irradiation (250 µE m^{-2} s^{-1}), as provided by unfiltered light, was evidently detrimental to algal growth and lipid productivity due to photo-inhibition. Reducing the light intensity to below (250 µE m^{-2} s^{-1}) enhances the algal growth, corroborating outputs from a previous study [30].

Acknowledgments

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