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Effects of different light spectra on the growth, productivity and photosynthesis of two acclimated strains of *Nannochloropsis* sp.

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Abstract

Light (quantity and quality) is the main growth limiting factor of photoautotrophic microalgae. The integration of selective permeable photovoltaic filters above microalgae cultivation systems has been proposed previously to improve both production efficiencies and economics. In order to optimize such system, we evaluated the growth and photosynthesis of two spectrally acclimated strains of *Nannochloropsis* sp. (MUR 266 and MUR 267) grown semi-continuously under different light spectra in this study. No significant differences in biomass productivity were observed between cultures acclimated under full blue (BL, 400-525nm), and narrow blue (LEDB, 430-490nm) light when compared to the positive control of white light (WL, 400-700nm) while lower values were recorded under red (RL, 600-700nm) and pink light (PL, 400-525, 600-700nm) for both species. When compared to WL, the photosynthetic performance (F_q'/F_m', αETR, ETR_{max}) of both species was higher under both BL and LEDB except for the F_q'/F_m' of MUR 267 under LEDB. Chlorophyll *a* content was highest in cultures acclimated to RL while values tended higher under LEDB, RL and PL for MUR 267. Total lipid yield of both MUR 266 and MUR 267 was higher under BL and PL respectively than WL. Based on the results of this study, theoretical modelling of the proposed photovoltaic-microalgae system indicate approximately 150-210 W m^{-2} of electricity could be potentially generated if only blue wavelengths (BL and LEDB) are selectively filtered from sunlight while converting the remaining unused spectrum of sunlight into electricity.

Abbreviations: PV, photovoltaic; PAR, photosynthetic active radiation; P-I, photosynthesis-irradiance curve; α, initial slope of the ETR based P-I curves; ETR, absolute electron transport rate; ETRmax, maximum electron transport rate of ETR versus Irradiance curve; PSII, photosystem II; RLC, rapid ETR versus Irradiance curve; F_q'/F_m', maximum photosynthetic light use efficiency of the open PSII in light-adapted state; LHC, Light harvesting complex.

1.0 Introduction

Among many growth parameters, the availability of light (quality and quantity) is by far the main limiting factor affecting the growth and productivity of any nutrient replete microalgae culture (Mandalam and Palsson 1998; Simionato et al. 2011). Incoming sunlight is composed of different wavelength bands such as ultraviolet (10-400nm), visible light (400-700nm) and infrared (700-1000nm). However, only the photosynthetic active radiation (PAR region, 400-700nm), constituting 43.8% of the entire solar energy is absorbed and utilized by autotrophs (Haxo and Blinks 1950; Moheimani and Parlevliet 2013).

Although in theory, all incident photons of PAR can be used for photosynthesis, not all these photons are equally absorbed and utilized by microalgae (Moheimani and Parlevliet 2013). Photosynthetic pigments directly govern the ability of algal cells in absorbing and utilizing specific photons (Kirk 1994). Therefore, algal light absorption and conversion efficiency is species specific and can significantly vary due to inherent properties such as the pigment profile, cellular architecture and chloroplast arrangement (Moheimani and Parlevliet 2013).

As illustrated by Keeling (2013), based on the evolutionary history of microalgal pigments, blue ($\approx$ 420-470nm) and red ($\approx$660) light are seen to be the most preferred spectral choice for the growth of microalgae when compared to other spectra such as green and yellow. In

71   addition, remaining underutilized wavelengths such as infrared (IR) and ultraviolet (UV) can

72   be detrimental to algal cells, either by increasing culture temperatures (Xue et al. 2005) or by

73   causing irreversible damage to photosynthetic proteins (Zeebe et al. 1996)

74   Therefore, in order to significantly improve the viability and efficiency of commercial scale

75   microalgae production facilities, substantial research efforts are still required for growth

76   optimization. Moheimani and Parlevliet (2013) proposed the integration of spectrally

77   selective photovoltaic (PV) filters above mass microalgae cultivation units to improve both

78   biomass yield and solar conversion efficiencies. Through such a system, wavelengths of

79   sunlight most efficient for the growth and photosynthesis of the selected microalgae would be

80   transmitted to the culture through the photovoltaic-filter apparatus while remaining unused

81   wavelengths; including IR and UV would be captured and converted to electricity (Vadiveloo

82   et al. 2016a).

83   The biomass productivity and photosynthesis of spectrally acclimated *Nannochloropsis* sp.

84   (MUR 266) was found to be enhanced under filtered blue (400-525nm) and pink light spectra

85   (400-525nm and 600-700nm) respectively (Vadiveloo et al. 2015; Vadiveloo et al. 2016b).

86   However, these studies were performed and standardized based on the concept of filtering

87   incoming sunlight as required by the PV-microalgae system where there were changes in

88   both radiant flux (light irradiance) and spectral quality. As such, both the variation in spectra

89   quality and irradiance were seen to affect the results of these previous studies. Therefore, in

90   order to only elucidate the effect of spectral quality and optimize the proposed PV-

91   microalgae system, there is also a need to study the effect of various light spectra without

92   changes in irradiance. The current study was carried out to determine the effects of different

93   light spectra including narrow wavebands of blue light with the same amount of radiant flux

94   on the growth, photosynthesis and biomass productivity of two strains of spectrally

95   acclimated *Nannochloropsis* sp. (MUR 266 & 267). The objective of this study was to

96 identify the most productive light spectra for both these strains of *Nannochloropsis* sp. for

97 future application in the PV-microalgae system.

98 The microalgal genus of *Nannochloropsis* is widely sought after due to its ability in

99 synthesizing large amount of lipid bodies and also for its high biomass productivity, making

100 it an ideal choice for the production of food, feed, nutraceuticals and also biofuel (Gouveia

101 and Oliveira 2009). However, significant variation in pigment expression has been reported

102 between different species/strains of *Nannochloropsis* resulting in changes of optical

103 properties and wavelength-specific absorption of cells (Millie et al. 2002; Tamburic et al.

104 2014). Thus, two different strains of *Nannochloropsis* with dissimilar pigment concentration

105 were used in this study to identify if physiological differences between strains may cause

106 discrepancy in light spectra absorption resulting in changes in growth and productivity.

107

108 **2.0 Materials and Methods**

109 **2.1 Microalgae strain and culture condition**

110 The marine eustigmatophyceae, *Nannochloropsis* MUR 266 (3.5 ± 0.5 μm in diameter) was

111 isolated from Rottenest Island, Western Australia 32.0062° S, 115.5123° E) while MUR 267

112 (3.5 ± 0.4 μm in diameter) was isolated from Swan-Canning Estuary, Western Australia,

113 32.0001° S, -115.8300° E). Both strains were obtained from the culture collection of the

114 Algae R&D Centre, Murdoch University. Both strains were grown in 500mL Erlenmeyer

115 flask with a working volume of 250mL and a start cell density of 1.5×10^7 cells mL^{-1}. The

116 inoculum cultures were maintained at 12:12 light:dark cycle and at a controlled temperature

117 of 25 ± 1°C by the culture room air conditioner unit. *Nannochloropis* sp. MUR 267 grown

118 under white light (100 μmol photon m^{-2} s^{-1}) was found to have significantly higher

119 chlorophyll *a* and accessory pigment content when compared to *Nannochloropsis* sp. MUR

120 266 (Vadiveloo et al., 2016b). Both strains of *Nannochloropsis* sp. were cultivated in

121 charcoal filtered (50 μm) and autoclaved natural seawater (Hillary's Beach) at 33 g L^{-1} NaCl

122 salinity. The seawater was enriched using F/2-Si medium nutrients as formulated by Guillard

123 (1975).

124 **2.2 Cultivation Setup**

125 Illumination with the different light spectra was achieved by positioning culture flasks inside

126 customized light boxes with their front panels either covered by coloured acetate filters or

127 subjected to the direct radiation of LED lamps (Vadiveloo et al. 2015). The inside of each

128 individual light box was dark covered (black) to prevent any reflection of light. The selected

129 filters (LEE Filters) were LEE 026 Bright Red (RL), Lee 363 Medium Blue (BL) and LEE

130 128 Bright Pink (PL) and these filters were illuminated by a Verbatim PAR 38 Cool White

131 LED lamp (Verbatim LED Lighting). The narrow band of blue light (LEDB) was supplied

132 using a Plusrite LED Floodlight (AU01-FL50 W/G/RGB, Plusrite AUSTRALIA PTY LTD).

133   To provide experimental control for the treatments, both strains of *Nannochloropsis* were

134   also grown under the full PAR spectrum of white light illuminated by the Verbatim PAR 38

135   Cool White LED lamp (positive control).

136   Sufficient open space was provided on top of each light box for ventilation (temperature

137   control), sample extraction and media renewal. Cultures were grown at 25 ± 1 °C controlled

138   by the temperature unit of the culture room. The temperature inside of the light boxes was

139   regularly measured and no difference was found between the temperature inside the light

140   boxes and the culture room. The mixing of cultures was ensured by using an 80 mm magnetic

141   stirrer with a mixing speed of 150 rpm. The light:dark cycle was standardized as 12 h:12 h.

142   Three independent replicates were carried out for each treatment and the standard error of

143   mean was calculated.

144   The incident photon-flux density of PAR on the surface center point of each culture flask was

145   standardized to 100 ± 10 μmol photons m^{-2} s^{-1} using a Li-185B quantum meter equipped with

146   a PAR quantum sensor, Li-190SB (LI-COR, USA) to ensure all cultures received the same

147   input (radiant flux) of light energy at its surface level. The spectral distribution of each light

148   condition was measured using a BLACK-Comet CXR-SR-50 Spectrometer (StellarNet,

149   USA) and is illustrated in Fig. 1.

150   **2.3 Growth Measurement**

151   All cultures were maintained in semicontinuous growth mode and acclimated to each light

152   spectra for at least 22 days prior to any measurements (two weeks batch and two weeks

153   semicontinuous). Cellular concentration was measured daily using a hemocytometer and was

154   used to calculate the specific growth rates of cultures in each condition. During the

155   semicontinuous growth, 50% of cultures (125mL) were harvested and renewed with fresh

156   medium every time the cultures reached 90% maximum cellular density ($\approx 6.5 \pm 0.54 \times 10^7$

157   cells mL^{-1}). Harvested samples were immediately used for biomass and photosynthetic

158   measurements. The specific growth rates (μ) and biomass productivity using the ash-free dry

159   weight procedure (i.e. gram of organic weight (AFDW) L^{-1} d^{-1}) under each light spectra were

160   calculated according to methods previously described by Moheimani et al. (2013).

161   **2.4 Chlorophyll and total lipid determination**

162   Chlorophyll *a* concentration was determined according to the methods of Jeffrey and

163   Humphrey while total lipid yield during each harvest was measured according to the protocol

164   by Bligh and Dyer as detailed in Moheimani et al. (2013).

165   **2.5 Saturation pulse based chlorophyll *a* fluorescence measurements**

166   Chlorophyll fluorescence measurements were performed using a WATER-PAM fluorometer

167   (Walz GmbH, Germany), consisting of a PAM-CONTROL unit and a WATER-ED

168   (emmiter/detector) unit. The WATER-ED unit consisted of LED's which provided the non-

169   actinic measuring light (spectral peak at 650nm), actinic light/saturation pulse (spectra peak

170   at 660nm) and far-red light (spectra peak at 730nm). The maximum quantum yield in light

171   (F_q'/F_m') of harvested samples were evaluated and calculated using the saturation light

172   method ($\approx$ 3500 μmol photons m^{-2} s^{-1}) as described in in Cosgrove and Borowitzka (2006).

173   The rapid light curves (RLCs) were performed with a 10s actinic exposure duration to each of

174   eight pre-set incremental irradiances (43, 651 96, 147, 218, 333, 499 and 709 μmol photons

175   m^{-2} s^{-1}) followed by a saturation pulse that lasted for 0.8s. Samples harvested from each light

176   condition were rapidly transferred to the ED-unit, followed by immediate measurements. A

177   fresh sample was used for each replicate measurement (minimum of 3).

178   Absolute electron transport rates (ETR) were calculated using the quantum yield values of

179   PSII (Φ_{PSII}) derived from the RLCs and the numbers of absorbed photon per chlorophyll-*a*

180   per second according to the equation ETR= $\Phi_{PSII} \times Q_{phar} \times 0.5$ (Wagner et al. 2006) where

181   Q_{phar} is the amount of photosynthetic usable radiation (PUR) absorbed by the microalgae

182   while 0.5 represents an assumption that radiant energy is divided equally between PSI and

183   PSII (Gilbert et al. 2000). Q_{phar} was calculated as described in Wagner et al. (2006) using the

184   incident spectra of each light treatment and the chlorophyll *a*- specific *in vivo* absorption

185   coefficients of corresponding samples measured using a StellarNet Inc IC2 integrating sphere

186   attached to the StellarNet Spectrometer, Model BLACK-Comet CXR-SR-50. These ETR

187   values obtained were then plotted against calculated Q_{phar} values to represent photosynthesis-

188   irradiance curve. The plotted curves were subsequently modelled and fitted with a best fit

189   curve through the waiting-in-line model function as suggested in Ritchie (2008). The model

190   assisted in deriving fitting parameters such as ETR_{max} and αETR which were then used to

191   estimate the photosynthetic efficiency of samples. Changes in the curve based parameters

192   (between treatments) were considered significant if the 95% confidence intervals were not

193   overlapping (Wagner et al. 2006).

194   **2.6 Statistical Analysis**

195   All experiments were carried out with a minimum of three replicates. The results are

196   expressed as arithmetic means ± standard error (SE). Significant differences in specific

197   growth rates, biomass productivity (organic dry weight), photosynthetic parameters, lipid and

198   chlorophyll *a* content were compared using One-Way Repeated Measure (RM) Analysis of

199   Variance (ANOVA) followed by the post hoc test of Holm-Sidak. Significance was based on

200     $P < 0.05$. All statistical analysis was performed using SigmaPlot version 12.5 for Windows.

3. Results

3.1 Specific growth rate and biomass productivity.

As illustrated in Fig. 2, no significant differences were observed in the specific growth rate (μ) of both MUR 266 and MUR 267 when grown semi-continuously and acclimatized under the different light spectra. Although growth rates are important in evaluating the progress of a culture, the most relevant parameter used to assess the potential of any commercial scale cultivation system is the biomass productivity. No differences in biomass productivity were observed between cultures grown under full blue light (BL), narrow blue light (LEDB) and white light (WL) for both *Nannochloropsis*, MUR 266 and 267 (Fig. 2). However, lower values were recorded for both strains acclimatized under pink (PL) and red light (RL) when compared to the positive control of WL (Fig. 2).

3.2 Chlorophyll *a* and total lipid yield

Chlorophyll *a* content of MUR 266 during each harvest was found to be higher under RL than the other treatments while values tended to be higher for cultures acclimated under PL, RL and LEDB for MUR 267 with no significant differences observed among them (Fig. 3). Total lipid yield (based on similar cell density during each harvest) was higher in cultures grown under BL and PL for MUR 266 and MUR 267 respectively when compared to the positive control of WL (Fig. 3).

3.3 Maximum Quantum Yield in Light

Fig. 4 illustrates the maximum quantum yield in light (F_q'/F_m') of both MUR 266 and MUR 267 when acclimatized under the different light spectra. F_q'/F_m' values were found to be higher under BL and LEDB than the other treatments for MUR 266 while values tended to be higher under WL, BL and RL for MUR 267 with no significant differences noted among them.

3.4 Fluorescence based ETR Curves

The initial slope (α) recorded from the plotted ETR against Q_{phar} curves for both MUR 266 and MUR 267 tended to be higher under BL, LEDB and RL when compared to the control of WL and also PL (Fig. 4). The photosynthetic capacity (ETR_{max}) of cultures was found to be highest under LEDB followed by BL for both MUR 266 and MUR 267 (Fig. 4).

4.0 Discussion

4.1 Biomass Productivity

Although many recent studies have looked into the response of various microalgae to different light spectra, almost none of these studies have been performed on cultures acclimated to the incident spectral conditions or on cultures maintained in continuous growth mode in order to eliminate the effects of constantly changing parameters such as cell density, growth rate, light penetration and also nutrient content (Das et al. 2011; Ra et al. 2016; Teo et al. 2014). As highlighted earlier, the main aim of this applied study was to determine the growth response of two strains of *Nannochloropsis* acclimated and grown semi-continuously under different light spectra when supplied irradiance was kept constant. The objective of this study was to identify the most efficient light quality which is able to enhance biomass productivity, biochemical synthesis and photosynthetic performance of both the selected *Nannochloropsis* sp. in comparison to the full spectrum of white light for further application in the proposed PV-microalgae integrated system. This study does not aim to compare the outcome of both strains of *Nannochloropsis* in respect to each other. It is also important to note here that although the white light treatment (WL) used in this study was found to have visible lower emission peaks in the blue-green region (490nm, Fig. 1), it still compromised of the entire PAR region (400-700nm) and was only used as a proxy to incident sunlight.

251   The higher biomass productivity of *Nannochloropsis* recorded under BL and LEDB for both

252   MUR 266 and 267 when compared to RL and PL were in accordance with many previous

253   studies (Vadiveloo et al. 2015; Teo et al. 2014; Das et al. 2011). In any photoautrophic culture

254   of microalgae, variation in biomass productivity between cultures under different

255   illumination conditions is directly governed by the efficient capture of light energy and its

256   correlation with carbon fixation (Atta et al. 2013). The increase in biomass productivity

257   recorded under both treatments of blue light (BL and LEDB) in this study correlates well with

258   the ability of *Nannochloropsis* in absorbing wavelengths of blue light more efficiently than

259   other wavelengths of light such as red (Tamburic et al. 2014). The added ability of blue light

260   in stimulating gene transcription while also enhancing the regulation of various activated

261   enzymes and the synthesis of DNA and RNA in microalgae cells might have also attributed

262   to the increase in biomass productivity (Ruyters 1980; Wallen and Geen 1971).

263   **4.2 Chlorophyll *a* and total lipid yield**

264   Acclimation to different light spectra has been well observed to bring forward changes in the

265   photosynthetic apparatus of microalgae such as the distribution and concentration of

266   chlorophyll *a* and other photosynthetic accessory pigments (Sánchez-Saavedra and Voltolina

267   1996; Vesk and Jeffrey 1977; Sánchez-Saavedra and Voltolina 2002). The higher chlorophyll

268   *a* content recorded under RL for MUR 266 and under RL, LEDB and PL for MUR 267 is

269   believed to be a response of chromatic photoacclimation in which the concentration of major

270   light harvesting pigments of *Nannochloropsis* such as chlorophyll *a*, violaxanthin and

271   vaucheriaxanthin esters with complementary absorption patterns to that of the incident light

272   spectra is regulated (Mouget et al. 2004, Lubián et al. 2000). Although chromatic

273   photoacclimation is well reported in cyanobacteria, this distinct phenomenon has also

274   observed to occur in other microalgae groups such as Chlorophyta and to a lower extent in

275 some diatoms in which the response of these groups are seen to vary and are taxon-specific

276 (Humphrey 1983; Mouget et al. 2004; Wallen and Geen 1971).

277 In the case of *Nannochloropsis* which only contain chlorophyll *a*, with violaxanthin and

278 vaucheriaxanthin esters as its other main light harvesting pigments, the in vivo absorption

279 peaks of these pigments are found to be under the blue (440nm for chlorophyll *a* and 490nm

280 for violaxanthin) and red (630nm and 675nm for chlorophyll *a*) portions of the visible light

281 spectrum (Gitelson et al. 2000; Owens et al. 1987). It is also important to note that changes in

282 pigment concentration is species or strain-specific and can vary due to chromatic

283 photoacclimation, explaining the discrepancy in outcome observed between both

284 *Nannochloropsis* MUR 266 and MUR 267 (Falkowski 1980; Prezelin and Matlick 1980).

285 The enzymes carbonic anhydrase and ribulose biophosphate carboxylase/oxygenase

286 (Rubisco) are vital for the regulation of carbon dioxide in microalgae (Teo et al. 2014). These

287 requisite enzymes which are also responsible for the production of triglycerides have been

288 observed to be under the direct control of blue light (Roscher and Zetsche 1986). Thus, an

289 increase in these enzyme activities stimulated by the illumination of monochromatic blue

290 light correlates well with the higher total lipid yield of MUR 266 (Roscher and Zetsche

291 1986).

292 The difference in lipid yield between both strains of *Nannochloropsis* (MUR 266 and

293 MUR267) can be possibly related to variation in morphological and physiological conditions

294 (Wahidin et al. 2013). Various factors such as light spectra, irradiance level and the nature of

295 microalgal photochemical machinery affect the photosynthetic response and the accumulation

296 of organic matter in various microalgae (Sánchez-Saavedra et al. 2016). These variables

297 govern the biomass production and overall biochemical concentration of algal cells (Sánchez-

298 Saavedra et al. 2016; Muller-Feuga et al. 2003).

4.3 Photosynthesis

The changes in the maximum quantum yield of PSII in light adapted samples (F_q'/F_m') is a valuable indicator of the instant physiological condition of a particular organism and represents the effectiveness of the open PSII reaction centers in capturing excitation energy (Genty et al. 1989). The higher F_q'/F_m' values recorded under both BL and LEDB when compared to the other treatments is believed to be an outcome of multiple factors. Previously, Das et al. (2011) reported that photons of shorter wavelengths such as blue light have greater possibility in striking the light harvesting complex (LHC) of microalgae such as *Nannochloropsis* resulting in higher photosynthetic efficiency. Building up on this, Tamburic et al. (2014) successfully measured the action spectrum of *Nannochloropsis* sp., illustrating the photosynthetic response of the microalga to different wavelengths of light. They concluded that shorter wavelengths of blue light (414nm) were absorbed more efficiently and transferred more effectively to PS II when compared to red light (679nm) at light intensities below the saturation threshold, resulting in higher photosynthetic rates under monochromic blue light (Tamburic et al. 2014). In addition, the acclimation of various microalgae such as diatoms to monochromatic blue light has also been reported to increase the photoprotective potential of cells (Costa et al. 2013; Brunet et al. 2014). This outcome is deduced by the ability of blue light in enhancing the capacity of non-photochemical quenching in algal cells while also bringing forward an increase in the pool size of xanthophyll cycle pigments when compared to red and white light of the same irradiance (Costa et al. 2013; Brunet et al. 2014).

Rapid light curves represent a non-destructive evaluation of the light response of PSII activity by providing important information regarding the saturation characteristics of electron transport and the overall photosynthetic performance including the photoacclimation ability of an organism (Ralph and Gademann 2005; Serôdio et al. 2006). The higher initial slope (α) recorded from the ETR curve of MUR 267 under LEDB, BL and RL for both species of

324 *Nannochloropsis* is brought forward by an increase in the efficiency of both light absorption

325 and energy conversion under these treatments (Henley 1993). On the other hand, the

326 remarkable increase in photosynthetic capacity (ETR_{max}) under blue wavelengths (BL and

327 LEDB) can be associated with the overall content and activity of the Calvin cycle enzyme,

328 Rubisco. An increase in Rubisco activity coupled together with the stimulation of the

329 photosynthetic electron transfer chain reactions by blue wavelengths have been previously

330 credited for an increase in the photosynthetic capacity of microalgae (Voskresenskaya 1972).

331 **4.4 Significance of this study**

332 Although no statistical differences were observed in the biomass productivity between WL,

333 BL and LEDB, all photosynthetic parameters measured (except for the F_q'/F_m' of MUR 267)

334 inclined to be higher under BL and LEDB when compared to WL for both species of

335 *Nannochloropsis*. In addition, this study also demonstrated visible changes in the biochemical

336 composition of *Nannochloropsis* such as the chlorophyll *a* content and total lipid yield when

337 acclimated under different light spectra. Blue light (BL) was found to significantly enhance

338 the lipid yield of *Nannochloropsis* MUR 266 On the other hand, no differences were

339 observed in lipid yield of *Nannochloropsis* MUR 267 when grown using PL, RL, LEDB and

340 BL. In terms of chlorophyll *a* content, RL was found to be most appropriate wavelength for

341 *Nannochloropsis* MUR 266 while LEDB, RL and PL were seen to bring forward the same

342 outcome for MUR 267.

343 Overall, except for the chlorophyll *a* content, it can be safely summarized that both blue light

344 treatments (BL and LEDB) irrespective of their wavelength band were as efficient as the

345 multichromatic WL and certainly more efficient than the other spectral qualities such as RL

346 and PL in enhancing the growth, lipid yield and photosynthesis parameters of both strains of

347 *Nannochloropis* sp. when radiant flux is kept constant.

348 Building up on this outcome, we successfully modelled and calculated the amount of

349 electricity that could be potentially produced through the proposed PV-microalgae system

350 according to the blackbox filter model as proposed by Parlevliet and Moheimani (2014).

351 Considering, no spectrally selective PV cell is currently available for the purpose of

352 calculating the potential electricity co-generation; LEE filters were used as proxy black box

353 filters for system evaluation. This theoretical model used was customized to determine the

354 maximum amount of electricity that could be generated if only blue wavelengths (BL and

355 LEDB) of incoming sunlight were filtered and channelled to the microalgae culture while

356 converting the remaining of the solar spectrum into electricity with minimal energy loss.

357 These modelling figures were calculated using the spectral photoresponse and light

358 conversion ability of highly efficient crystalline silicon solar cells when they are provided

359 with a narrow spectrum of incident light.

360 In this case, they were modelled to be receiving the light that was not transmitted to the

361 microalgae by both the BL and LEDB treatments. On average, a total of 431 W m^{-2} of solar

362 energy in the PAR region is directly available to microalgae cultures in the locality of

363 Western Australia if no PV/filter is placed above the algae pond (Parlevliet and Moheimani

364 2014). However, in an outdoor scenario, if incoming sunlight was to be filtered and only

365 selective parts of the light spectrum such as BL of and LEDB used in this study were to be

366 supplied to the microalgae culture using the proposed PV/filter device. A maximum of 151

367 Wm^{-2} (for BL) and 210 Wm^{-2} (for LEDB) of electrical energy could be potentially generated

368 by the modelled PV system from the remaining unused portions of sunlight (i.e. green, red

369 and yellow spectrum of sunlight).

370 The amount electricity generated by the PV apparatus is more than sufficient for the

371 operating mechanism of the microalgae cultivation system (e.g., pumps and paddlewheels)

372 while also providing a viable option for powering extra artificial lighting (i.e. Blue or/and

373 Red LEDs) if required by the microalgae. This study also demonstrates that by narrowing the

374 incident spectra of light supplied to the microalgae, an increased in the amount of electricity

375 co-generated from the modelled system is achieved without any compromise to the growth

376 and yield of the microalgae culture.

377 Although the exact system for dividing the incident light spectrum for both purposes is not

378 fully determined yet, several existing options can be potentially optimized to match the

379 spectral distribution of BL and LEDB used in this study while channelling the remaining

380 unused wavelengths into electricity. Among them include the use of specifically tailored

381 semitransparent thin film PV, luminescent dollar concentrators which allow selective

382 incoming photons with insufficient excitation energy to pass through while capturing and

383 converting portions of the remaining photons with sufficient excitation energy into electricity

384 and most interestingly the use of spectrally selective glass panels composed of insulated

385 glazing units that can transmit arbitrary wavelengths of visible light while capturing and

386 converting portions of IR and UV into electricity (Debije and Verbunt 2012; Moheimani and

387 Parlevliet 2013; Rosenberg et al. 2014). In addition, the recent development of high

388 performance PV cells which can successfully perform spectrum splitting on a small scale

389 using dichroic mirrors and optics is of great value (McCambridge et al. 2011). A similar,

390 albeit expensive system, could be built to match the spectral distribution of blue wavelengths

391 used in this study or approach the ideal system through selective thin fill deposition

392 (McCambridge et al. 2011).

393 Moreover, the results of this study would be of great use in aquaculture facilities where the

394 nutritional content (i.e. lipid) of microalgae such as *Nannochloropsis* could be enhanced

395 using specific light spectra (i.e. blue light) for the feed of rotifers and fish/prawn larvae.

396 Although lipids are important elements required as part of the diet of microalgae, the fatty

397 acid composition and concentration among many others actually determines the nutritional

398 value of microalgae in the aquaculture industry. Therefore, further in depth studies looking

399 into the effects of different light spectra on the fatty acid composition of spectrally acclimated

400 of *Nannochloropsis* would be of great value and interest. As observed with this study,

401 variation in light spectra can bring forward significant physiological changes affecting

402 growth, biochemical composition and the nutritional value of algal cells (Sánchez-Saavedra

403 et al. 2016).

404 **5.0 Conclusion**

405 This study evaluated the growth, biomass productivity; biochemical composition and

406 photosynthetic performance of two strains of *Nannochloropsis* sp. (MUR 266 & MUR 267)

407 when grown under semi-continuous mode and acclimated under different light spectra. No

408 differences were observed in the biomass productivity of strains of *Nannochloropsis* sp. when

409 grown under blue light irrespective of the wavelength band (BL & LEDB) and white light. In

410 addition, blue light was also found to be most efficient for the photosynthetic performance of

411 both strains of *Nannochloropsis* sp. when compared to the other light spectra. Lipid yield and

412 chlorophyll *a* content was found to be enhanced under blue light and red light respectively for

413 MUR 266 while no significant differences were observed under pink, red and blue light for

414 MUR 267 for both parameters. By only providing blue light to the microalgae culture,

415 between 151-210 W m^{-2} of electrical energy could be potentially generated from the

416 remaining unused wavelengths of light. By narrowing the spectrum of light received by the

417 microalgae (i.e. LEDB), a larger proportional of incident sunlight can be diverted for other

418 purposes such as electricity production without negatively affecting the growth, biomass

419 productivity and photosynthetic performance of the cultures of *Nannochloropsis* sp..

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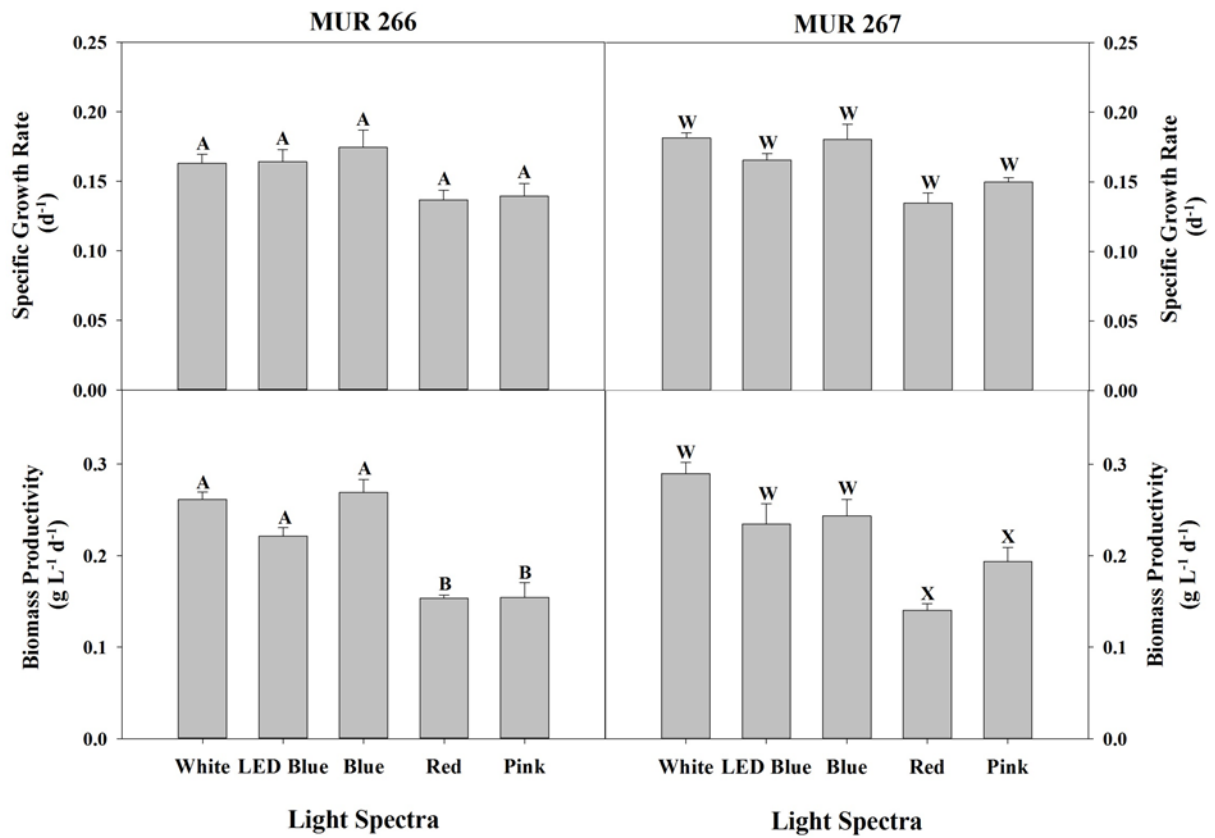
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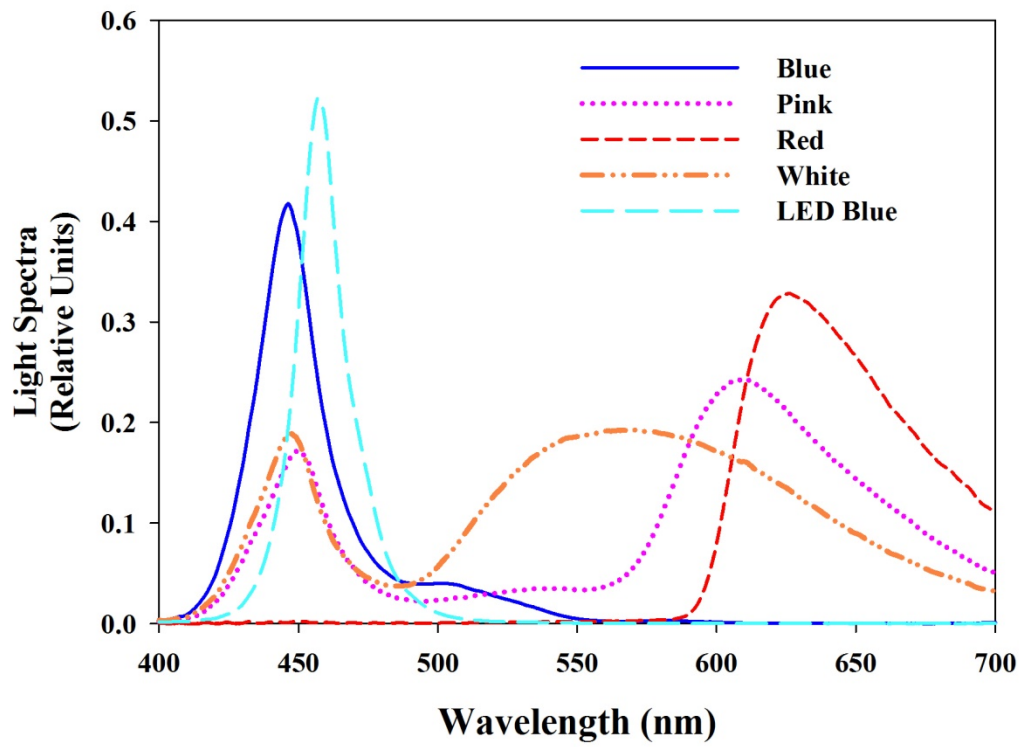
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613   **Fig. 1**: The relative distribution of each light spectra inside the customized light boxes.

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Fig. 2: Specific growth rate (μ) and biomass productivity (g AFDW L^{-1} d^{-1}) values for both

species of *Nannochloropsis* grown semi-continuously and acclimated under the different light

spectra (n=5 ± standard error). The same letter above each column indicates no significant

differences (One Way Repeated Measures ANOVA P > 0.05).

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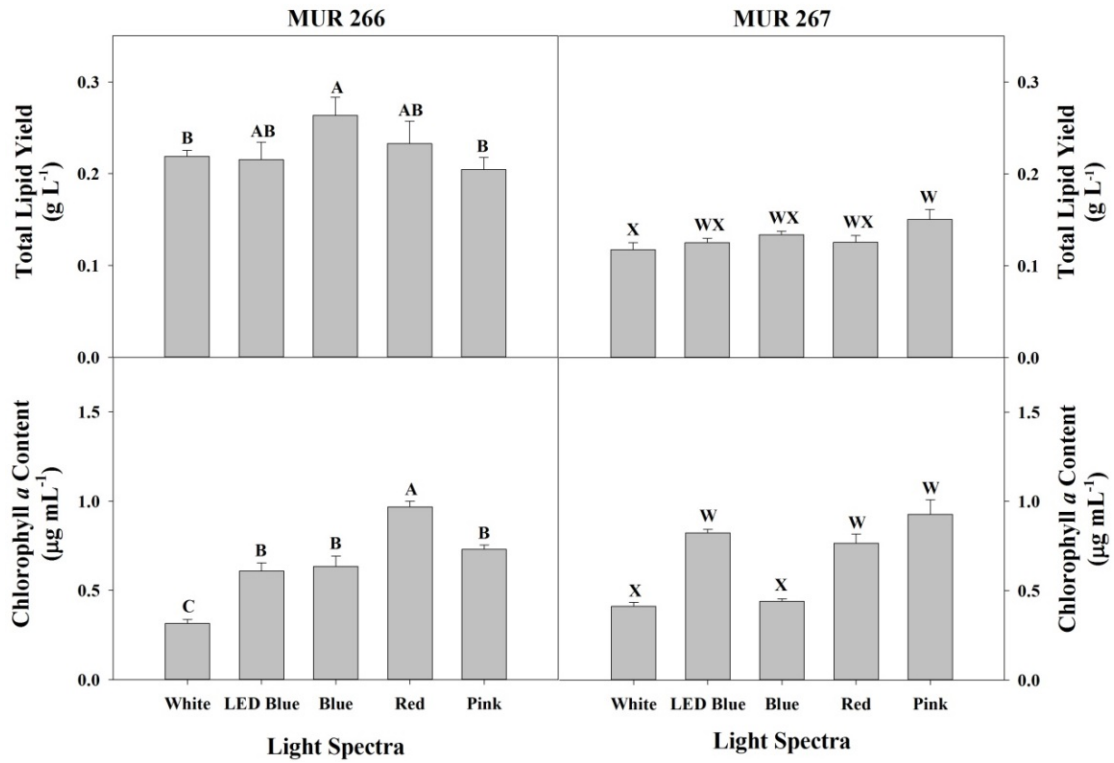
Fig. 3: The average chlorophyll *a* and total lipid yield during each harvest for both species of *Nannochloropis* acclimated under the different light spectra (n=5 ± standard error). The same letter above each column indicates no significant differences (One Way Repeated Measures ANOVA $P > 0.05$).

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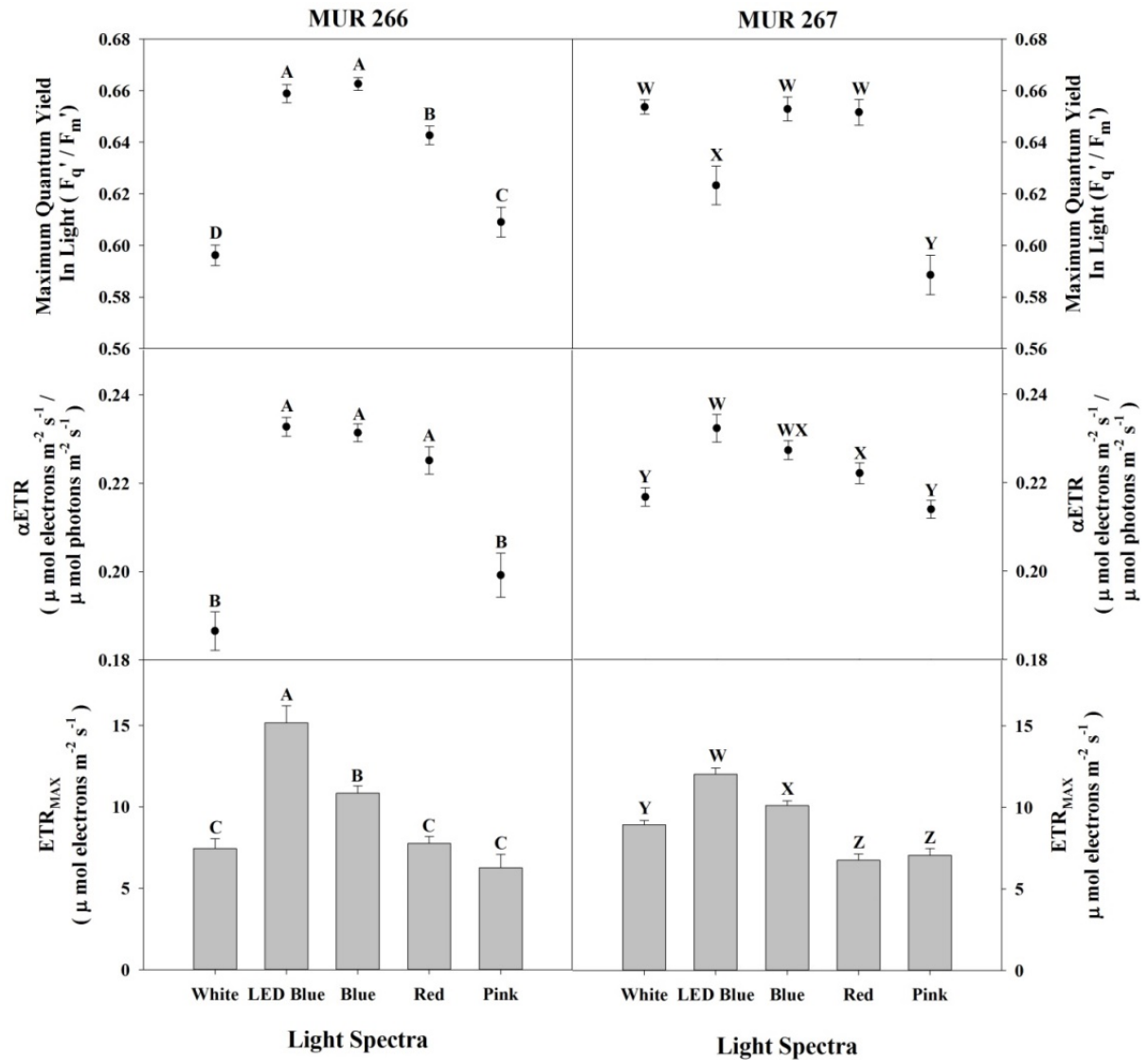
Fig. 4: Maximum quantum yield in light (F_q'/F_m') and the curve fitting parameters (α and ETR$_{max}$) derived from the chlorophyll fluorescence P-I curves of both MUR 266 and MUR 267 grown and acclimated under the different light spectra (n=5 ± standard error). The same letter above each column indicates no significant differences (One Way Repeated Measures ANOVA P > 0.05).

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