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Effect of Selected Light Spectra on the Growth of *Chlorella* spp. (Chlorophyta)

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Abstract

The possibility for simultaneous production of chemical and electrical energies from a single microalgae cultivation plant is opening a new chapter in the efficient use of resources to maximize biomass productivity. In the current study, the effect of selected monochromatic lights (blue, red and pink) from spectrally selective filters on the biomass productivity of semi-continuously grown *Chlorella* spp. was investigated under laboratory conditions using light emitting diodes (LEDs). The temperature variations inside of the customized light boxes containing cultures under different light spectra were significantly different ($p < 0.001$). Cell density of the alga under the different light treatments was not similar and ranked as White > Pink > Blue > Red. The biomass productivity was highest under the white light (60.07±9.38 mg L^{-1} d^{-1} dry weight, DW) and varied significantly ($p = 0.004$) among the treatments. However, productivities under the white (60.07±9.38 mg L^{-1} d^{-1} DW) and pink (56.25±9.85 mg L^{-1} d^{-1} DW) lights was statistically insignificant ($p = 0.551$). The result shows that biomass productivity of the alga, *Chlorella* spp., can be manipulated through targeted supply of specific spectral bands (e.g. pink light). Therefore, the remaining portions of the spectrum which are not utilized by the alga for growth can potentially be converted to electricity through a robust and highly efficient photovoltaic cell.

Keywords: Biomass productivity, *Chlorella* spp., Electricity, Light spectra, Photovoltaic
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Introduction

Microalgae have high potential for solving human food crises. There is also the potential to use microalgae as sustainable sources of animal feed, biopharmaceuticals, nutraceuticals, cosmeceuticals, and industrial biomaterials (Spolaore et. al., 2006) in order to combat malnutrition, hidden hunger and food shortage. Furthermore, microalgae are increasingly receiving attention as a promising feedstock for sustainable production of biofuel and bioenergy (Gouveia and Oliveira, 2009; Nwoba et. al., 2016) amidst a changing climate. Biodiesel are produced from the oil of the microalgal biomass, whereas bioethanol is made from the carbohydrate portion of the biomass (Chisti, 2007). In the same vein, biomethane is produced via anaerobic digestion of the spent biomass. These applications are eminent in a highly expanding global economy and era of burgeoning human population to ensure a sustainable economy. These applied research approaches including bioprocess engineering, fermentation, harvesting and downstream processing require mass-culture strategies. However, current conventional methods for large-scale microalgae production is limited by low cell density which results in low biomass productivity, thereby making production cost prohibitively high. This limitation is brought forward by a number of biotic, abiotic and operational factors (Cuello et. al., 2015).

Light is the most critical driver of photosynthesis in plants and has remained a very important factor limiting microalgae growth and productivity in photobioreactors. The spectral distribution of light (e.g. blue, red, yellow or pink) including quality and quantity control microalgae growth, productivity and photosynthetic efficiency (Tredici and Zittelli, 1998; Vadiveloo et. al.,

2015; Vadiveloo et. al., 2016; Zhu et. al., 2008). The photosynthetic efficiency of microalgae for converting light energy to chemical energy in carbohydrates is theoretically estimated at 12 % (Wilhelm and Jakob, 2011; Zhu et. al., 2008). This maximum theoretical limit is by far above the real life photosynthetic efficiency of 2 % of the sum solar spectrum (Vadiveloo et. al., 2016). It is worthy to mention that it's only lights in the wavelength range of 400–700 nm, which corresponds to the photosynthetically active radiation (PAR) and accounts for 47 % of the total solar spectrum (Gueymard, 2001) is utilized in photosynthesis by microalgae (Zhu et. al., 2008). This means that the photosynthetic performance of microalgae is not driven by the entire solar spectrum. The response of microalgae to spectral composition of light is specie-specific (Vadiveloo et. al., 2015). Hence, selecting monochromatic lights equivalent to or near absorption maxima of microalgae pigmentations would create an opportunity for harnessing the poorly absorbed spectra including ultraviolet (UV) and infrared (IR) for other profitable economic benefits such as generation of electricity.

Photovoltaic devices (PVDs) are used to convert solar energy to electrical energy and a maximum efficiency of 20 % has been reported from commercially available PVDs (Moheimani and Parlevliet, 2013; Vadiveloo et. al., 2016). The success recorded in the manufacture of industrial-scale spectrally selective photovoltaic devices (SSPD) has opened a new discourse for the utilization of monochromatic lights in microalgae cultivation (Moheimani and Parlevliet, 2013; Rosenberg et. al., 2014). This implies that portions of the PAR that are most suitable for growth and productivity of microalgae are allowed to pass through the SSPD for biomass production while the remainder is routed to a solar collector to generate additional energy. The economies of this concept are that through light filtration, reasonable portions of the solar electromagnetic spectrum that are incident on cultivation systems and poorly utilized by microalgae be transformed to electricity (Figure 1). The electricity generated can be used to drive microalgae production operations (e.g. harvesting, mixing and dewatering) and/or produce additional lightings to boost productivity (Parlevliet and Moheimani, 2014). For ease of optimization of the above process, there is need to evaluate the growth performance of desired species of microalgae under different wavelengths of light. Hence, the effect of different monochromatic (one wavelength) lights on the growth, productivity and metabolites production by specific microalgae species is considerably less understood.

Chlorella spp. (Chorophyta) is a priority microalga that is commercially grown for food, high value nutraceuticals, and biopharmaceuticals (Spolaore et. al., 2006). This is as a result of its excellent growth rate and biomass productivity as well as high potential as a raw material for bioenergy (Gouveia and Oliveira, 2009; Nwoba et. al., 2016). Taiwan Chlorella Manufacturing Co. (Taiwan) and Klöze (Germany) are currently the world leaders in *Chlorella* production, with an annual productivity of more than 2000 tons (Hallmann, 2007; Spolaore et. al., 2006).

In the current applied research, the impact of monochromatic lights obtained from different selectively-permeable filters of blue (400–525 nm), red (600–700 nm), and pink (400–525 nm and 600–700 nm) on the growth and productivity of *Chlorella* spp. was investigated in the laboratory using cool white lights (LEDs).

Materials and methods

Microalgae species, acclimation and culture condition.

The freshwater Chlorophyte, *Chlorella* spp. used in the current study was isolated from a freshwater dam at Ndiulo-Amike Ufuobodo (6.406˚N, 7.947˚E), Ebonyi State. The isolation and growth was carried out using Chu 13 medium (Yamaguchi et al., 1987). The microalga was grown in 500 mL conical flask at a culture volume of 200 mL. The alga was acclimated to the different light conditions prior to commencement of the experiment. The initial cell density at the beginning of experimental measurement was 12.05 x 10^6 cells mL^{-1}.

Experimental setup and cultivation mode

A customized rectangular polyvinylchloride (PVC)-based light boxes (90 x 24 cm, Length x Width) illuminated with three ultra-bright LEDs (Winzone lighting, WZ-01005, 6W) fitted on the PVC at distances of 15 cm apart (length) and 2 cm (from the base) were constructed and used for the study. The surface of the LEDs was covered with respective selective permeable filters, LEE 363 Medium Blue, LEE 128 Bright Pink, and LEE 026 Bright Red. The selection of the filters was based on availability and spectral

distribution (see Vadiveloo et. al., 2015 for details). The filtered lights were allowed to illuminate the culture flasks with no light contamination. In order to ensure adequate experimental controls for the treatments, *Chlorella* spp. was cultivated under the white light (i.e. without filters, full PAR spectrum) and in dark (completely covered). To ensure sufficient ventilation for control of temperature, openings were provided at the top of the customized boxes (Vadiveloo et. al., 2015). Opening was also made for sample collection. The cultures were grown in the laboratory at room temperature with intermittent manual shaking three times on weekdays. The daily temperatures recording inside of the customized boxes were determined manually at 08:00, 12:00, and 16:00 using a thermometer.

The experiment was conducted in three replicates for individual spectral treatment, with the cultures operated in semi-continuous system and light:dark regime; 10 hour:14 hour. This photoperiod was maintained through a constant supply of power from 2 x 12 V, 120 AH battery (to supplement electrical power) coupled to timers set at 6:00 a.m. to 4:00 p.m. Semi-continuous operation was carried out by harvesting 50 % (100 mL) of the culture volume and replenished with the same amount of fresh Chu 13 medium, every time maximum cell density was attained (Nwoba et. al., 2016). Prior to commencement of actual measurements, cultures were acclimated to individual light spectrum in the customized boxes for four weeks (2 weeks apiece for batch and semi-continuous operations) (Vadiveloo et. al., 2015).

Analytical methods

Culture samples from each light treatment was collected for determination of cell density at 10:30 am every second day. Biomass concentration (DW, dry weight), and productivity of the alga were determined at maximum cell density. The alga was collected on Milipore filter papers by filtration and washed with sterilised deionized water. The filter papers were folded into two and blotted gently to remove excess water. The filter papers were kept in small plastic bags in a closed container and stored at -5 °C in the dark until analysis (Nwoba et. al., 2016). Cell count (expressed as cells mL^{-1}) was determined using the Improved Neubauer Counting Chamber (Moheimani et. al., 2013). The biomass productivity (expressed as DW, mg L^{-1} d^{-1}) was calculated based on Moheimani et. al. (2013).

Statistical analysis

All experiments were done with at least three biological replications and results reported as means ± standard deviation (SD) over the experiment duration. One-Way Repeated Measures Analysis of Variance (RM-ANOVA) via a SigmaPlot software (Systat Inc., ver. 12.5) was used to determine differences among treatments. Significant differences were declared at 5% probability level and the Holm-Sidak method was used for testing significant differences in means. The cell density of the alga was log-transformed.

Results

The temperature profile on the inside of the customized light boxes was compared and shown in Figure 2. The treatment under red light showed a superior temperature variation (Figure 2b). The overall average temperatures observed in the customized light boxes were White, 30.94±1.95; Pink, 31.16±2.74; Red, 33.78±4.82 and Blue, 30.65±1.95 °C. It is therefore obvious from the values that maximum average temperature was found in the box under red light. Statistically significant differences (One-RM ANOVA, $F = 103.59$, $p < 0.001$) were detected in the mean temperature values of the treatments. However, a pairwise comparison procedures revealed no significant differences in the overall average temperatures of Pink and White lights (Figures 2a and b, $p = 0.289$) and, White and Blue light (Figures 2a and c, $p = 0.270$). Similar comparisons between White and Red (Figures 2a and b), Pink and Red lights (Figures 2b and d) showed significant differences between the treatments ($p < 0.001$, $n = 164$).

The experiment was started with an initial cell density of 12.05 x 10^6 cells mL^{-1} in all the treatments, including the culture not exposed to light. The variation in cell densities of the treatments under the different light spectra are shown in Figure 3. At the end of the batch phase, maximum cell densities were respectively; 24.64±1.88, 18.96±0.84, 26.40±1.52 and 30.93±0.71 cells mL^{-1} for Pink, Red, Blue, and White lights after 14 days cultivation time. Hence, maximum cell density was found in the culture under full PAR (White light) (Figure 3d). Since the stationary phases of the cultures were not significantly different as found during the acclimation stages (data not shown), culture harvest was a function of

white light on attainment of maximum cell density. Following the batch phase, the treatments were switched to semi-continuous operation, and regularly harvested based on 50 % harvest of the cell density for each light treatment. During the semi-continuous cultivation, the highest cell density was obtained under the White light (36.16±0.49 cells mL^{-1}) while the lowest was found under the Red spectrum (21.95±1.39 cells mL^{-1}) (Figure 3b and d). The overall average cell density for the entire cultivation period (batch plus semi-continuous) for each treatment differed significantly from one another (One-Way RM ANOVA, $F = 106.30$, $p < 0.001$). All pairwise multiple comparison procedures showed significant differences ($p < 0.001$) in temperature between treatments. When this alga was grown under no light (dark) condition, no net microalga growth was observed after four days cultivation period. Amazingly, the overall cell concentration (density) dropped significantly on subsequent measurements (Figure 4).

The total volumetric biomass productivity for this alga obtained in this study was highest under the White light (One-Way RM-ANOVA, $F = 9.28$, $p = 0.004$). Hence, the alga showed lowest biomass productivity (31.94±7.35 mg DW L^{-1} d^{-1}) under the Red spectrum (Figure 5). Interestingly, there was no significant differences (One-Way RM-ANOVA, $p = 0.55$) in the volumetric biomass productivity obtained under White and Pink lights (Figure 5). Similarly, no significant differences ($p > 0.05$) were observed in the productivity from Blue and Red spectra.

Discussion

Temperature is the second most critical factor after light that limits (or regulates) microalgae growth and productivity in photobioreactors. High and low temperatures outside the tolerance range of algae growth affect its performance in cultivation systems (Nwoba et al., 2016). The higher temperature observed under the red light (Figure 2) would be due to accumulated heat in the box due to emission (radiation) from the red light. Since there was net increase in the cell density of the alga in cultures under different light treatments, it follows that the alga tolerated the prevailing temperatures in the light boxes. Hence, the prevailing temperature did not exceed the tolerance range of the alga. Temperature therefore would not have been a limiting factor in the growth and productivity of the alga.

It has been reported that cell density of alga increases linearly with increase in irradiance and spectral quality (Wallen and Geen, 1971). The higher density of the alga obtained under the white light (Figure 3) is due to its positive impact on photosynthesis (Sorokin, 1958; Vadiveloo et al., 2016). The lower cell density under the blue spectra would be due to reduced irradiance from the blue filter.The cell density of the *Chlorella* spp. grown in the dark showed a significant decrease in cell number. This decrease was not unexpected because all photoautotrophic microalgae would require light as a source of energy for their growth.

The biomass productivity (mg DW or ash-free dry weight, AFDW L^{-1} d^{-1}) is the most relevant index associated with viable commercial-scale microalgae production facilities (Vadiveloo et al., 2015). Therefore, the variation in biomass productivity of the *Chlorella* spp. would not be attributed to a single factor since irradiance and light distribution, and temperature varied in the light boxes. Microalgae are known to increase their growth and productivity under increasing temperature (Ras et al., 2013). The algae would continue their growth until optimum temperature is attained, under which further increase in temperature above the tolerance limit would result to decrease in growth and productivity of the algae due to weakening of their structural integrity by heat stress. Heat stress impacts negatively on the growth and productivity of algae by inactivation of essential enzymes (Salvucci and Crafts-Brandner, 2004). The lower biomass productivity brought forward by the lower cell density of the alga observed under the Blue and Red spectra would be due to significant reduction in the transmitted energies. Vadiveloo et. al. (2015) had earlier reported lower growth rate in a culture of *Nannochloropsis* spp. grown under blue spectrum. The fact that higher productivity was obtained under the White light would have been influenced by spectral quality and quantity. Furthermore, the productivity obtained under the White light was statistically similar to Pink light (400 – 525 and 600 – 700 nm) (Figure 5). This outcome is in agreement with the findings of Vadiveloo et. al. (2016) who observed that multichromatic lights, Pink and White lights were highly efficient for photosynthesis by *Nannochloropsis* MUR 266

and MUR 267 spp. for biomass production. In terms of application, Parlevliet and Moheimani (2014) has already modeled this concept where selected portions of the available solar spectrum are transmitted to microalgae cultivation systems through selective filters as utilized in this study. It is assumed that the transmitted irradiance is to be directly incident on the microalgae facilities, while the remaining portions of the spectrum can be collected and redirected to a highly efficient photovoltaic cell with little or no loss of energy. From the findings of Parlevliet and colleague, up to 431 W m^{-2} of energy in the 400 – 700 nm of the solar spectrum is accessible to the algae if selective filters or photovoltaic devices are not positioned over the cultivation system. As pointed out earlier, the maximum efficiency of photosynthesis by microalgae is less than 5 % of the visible spectrum (in practical terms) (Moheimani and Borowitzka, 2011), hence, the entire PAR region is not efficiently absorbed and used by microalgae. Utilization of selective filters such as LEE filters (e.g. 026 Bright Red, 363 Medium Blue and 128 Bright Pink) in the cultivation of microalgae will reduce the incident PAR to 47 and 148 W m^{-2} for Red and Pink filters respectively without affecting the photosynthesis of the microalgae growth systems (Islam et. al., 2011; Moheimani and Parlevliet, 2013). The remaining unused portions of the solar spectrum (including UV and IR) can be collected, redirected and converted through a highly efficient photovoltaic cell to electricity. It is worthwhile to state that the portion of electromagnetic spectrum (light spectrum) to be directed to the cultivation systems for biomass production, to enable the remaining portions harnessed for electricity generation depends on (i) species of the microalgae, (ii) growth response, (iii) yield to different light spectra, and (iv) part of the spectrum that is vital to the growth of the microalgae (Moheimani and Parlevliet, 2013). The results of this research have demonstrated that pink spectrum is the most suitable source of photons for the growth and biomass productivity of *Chlorella* spp.

Conclusion

This study indicates that the full White (400 – 700 nm) and the Pink (400 – 525 nm and 600 – 700 nm) lights were the most efficient for conversion to biomass in the growth of *Chlorella* spp. Hence, commercial-scale production of *Chlorella* spp. (especially in outdoor cultures) can be done using selective pink photovoltaic filters for simultaneous biomass, chemical and electrical energy productions. This applied research approach would create the opportunity to diminish dependence on artificial lights and allow for optimal harnessing of the natural sunlight. Meanwhile, this would significantly diminish the cost of cultivation of microalgae with concomitant production of chemical and electrical energies from available sunlight.

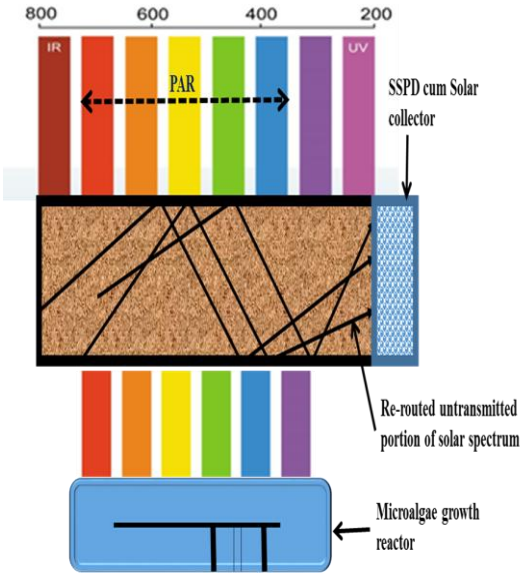

Figure 1: Schematic representation of the proposed novel selective photovoltaic device. The chosen wavelengths are passed to the microalgae culture through the selective filters and the unused portions of the incoming light routed to the photovoltaic cell for generation of electricity.

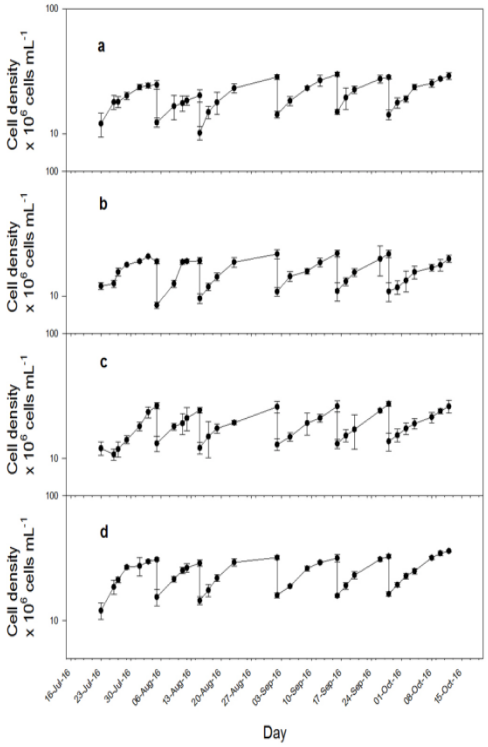

Figure 3: Common logarithm transformed cell density of *Chlorella* sp. grown under different light spectra of Pink (*panel a*), Red (*panel b*), Blue (*panel c*), and White (*panel d*) lights.

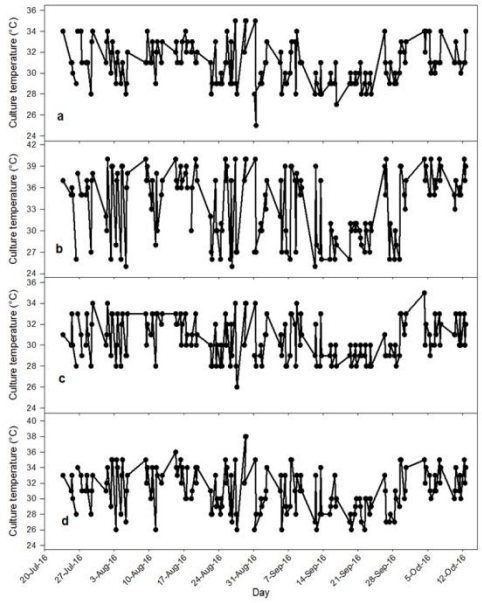

Figure 2: Temperature in the customized boxes containing cultures under different light spectra, (a) White, no filters, (b) Red, (c) Blue, and (d) Pink lights.

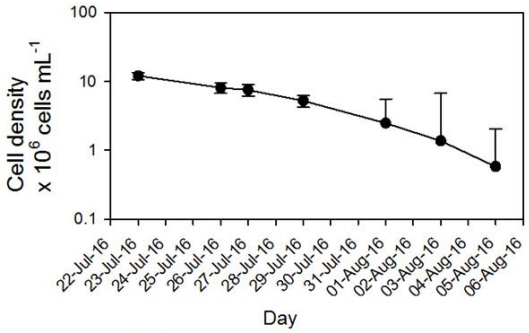

Figure 4: Common log transformed cell density of the *Chlorella* sp. grown in the dark

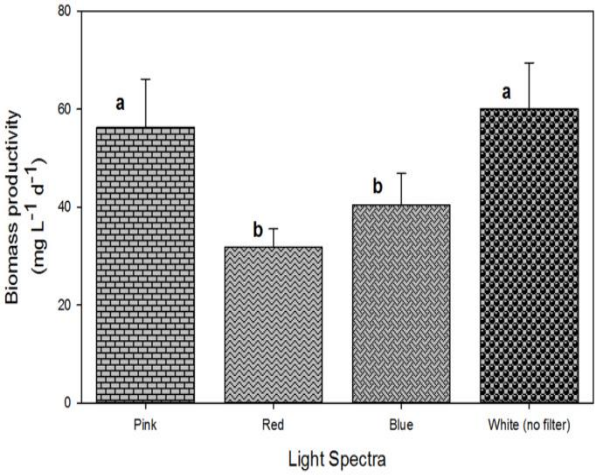

Figure 5: Volumetric biomass productivity (based on dry-weight) of *Chlorella* sp. grown under the different monochromatic lights. n = 5. The same letter indicates no significant differences (One Way Repeated Measures ANOVA, P > 0.05).

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