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Combined Effects of LED Lights and Chicken Manure on *Neochloris oleoabundans* Growth

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ABSTRACT

In this study a photobioreactor prototype is presented for the culture growth of microalgae model organism *Neochloris oleoabundans* by using chicken manure waste as feedstock along with the optimum combination of led light wavelengths and light intensity. Particularly interesting results are observed on the strains fed by chicken manure medium under the proper combination of red and blue LED light illumination, the microalgal growth resulted comparable with the strains fed by the costly commercial microalgal growth medium (BG 11 medium). Cell concentration, optical density, growth rate, cell size, total lipid and photosynthetic pigment content have been monitored during a time-course experiment. The data suggest that there are difficulties due to white light diffusion into the dark chicken medium, which leads to a generally lower intensity scattered along all wavelengths; blue or combined red and blue lights resulted in a higher irradiation density, affecting microalgae cell growth.

Keywords: Biowaste, chicken manure, light-emitting diodes, microalgae, *Neochloris oleoabundans*.

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1. INTRODUCTION

In recent years, the non-fossil energy consumption have received growing attention in many countries, particularly China (Zhang, 2016) and USA (Jonathan et al., 2016). The search for solutions aimed at carbon pollution reductions and public health improvement. In thıs scenario, microalgae offer an alternative for the production of sustainable fuels. The most common fuel obtained from microalgae is biodiesel, but there are other types of fuels such as bioethanol or biobuthanol (Ellis et al., 2012), biogas (Passos et al., 2014) and bio-oil (Kim et al., 2014) which can be obtained from the products of microalgae culturing. In addition, microalgae can be used also for secondary metabolite production: antioxidants such as fucoxanthin (Xia et al., 2013), astaxanthin (Hong et al., 2015) and β-carotene (Varela et al., 2015), but also vitamins (Grossman, 2016) and anticancer drugs (Borowitzka, 1995). They can even be considered as feed for aquaculture (Sirakov et al., 2015) or animal feed (Kotrbáček et al., 2015).

The challenge is to optimise a protocol to grow algae and eventually to process them in the most economical way. In general microalgae need a light/dark regime and appropriate light duration, intensity and wavelengths that are still being studied (Mallick et al., 2016). Inadequate light intensity may cause growth limiting or photo-oxidation and inhibition (Carvalho et al., 2011). Light limitation is one of the major factors affecting microalgal growth that can be tolerated by desaturation of chloroplast membranes (Mock and Kroon, 2002). However, increased light intensity over the saturating limits, causes photo-inhibition due to the disruption of the chloroplast lamellae (You and Barnett, 2004). For photosynthetic organisms, light is a limiting factor not only in terms of variations in its intensity due to the possible mutual shading of algal cells , but also the wavelengths can play a key role microalgal growth (Chen et al., 2011b). Many studies

focus on red and blue light applications (Schulze et al., 2014). It must be mentioned that the use of the efficient wavelengths application (illumination) depends on the algal species, according to the optimum growth parameters such as; the increase in growth rate or in cell dimensions, in secondary metabolite production, etc. Often this efficient illumination is obtained by means of adding LED-specific wavelengths on a background of fluorescent light (Ra et al., 2016) or manipulating solar wavelengths through the special filters (Michael et al., 2015).

Illumination via LED lights can be considered as an option in order to obtain high performance in terms of quality of monochromatic irradiation whilst achieving energy efficiency. Solid state semiconductor based light source is by far the most efficient radiative technology. The Philips Lumiled catalog published in 2012 indicates the typical efficiency for each colour which is derived by using the luminosity function. The typical efficiency for white LED is calculated on the basis of a wavelength value corresponding to the center of the visible spectra (555 nm) due to the fact that white light is not mono-chromatic, but it is a combination of multiple wavelengths, and it corresponds to a value of 0.22 W/W. Blue LED light source is proven to be the most efficient light source (0.35 W/W) with a lower cost. Looking at the typical absorption spectra of algae, the maximum peak of absorbance is located in the range of blue light, while a lesser peak is located in the range of the red (red light typical efficiency is 0.39 W/W).

Biochemical composition of the microalgae mainly depends on the growth conditions. There are four major types of growth conditions as photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic cultivation (Wang et al., 2014). During the photoautotprophic cultivation, microalgae produces chemical energy through photosynthesis by using light as an energy source and inorganic carbon as carbon source

(Huang et al., 2010). In heterotrophic growth microalgae uses organic carbons as carbon and energy sources in the absence of light (Chen et al., 2011a). During the heterotrophic growth it is well known that lipid accumulation is higher than in photoautotrophic growth and in contrast, photoautotrophic cultivation provides higher photosynthetic pigment content compared to heterotrophic cultivation.

Another economic consideration is the medium that is used for the algal cultivation. As it is already known, microalgae can be used for wastewater treatment in order to remove nitrogen, phosphorous, or metal ions (Pittman et al., 2011), coupling this environmental goal with microalgae based biofuel production. In this way, the advantage becomes doubled: on the one hand, this strategy helps the creation of a sustainable chain for waste management reducing the negative impact of pollutants on the environment; whilst on the other hand, converting waste into a resource for nourishing microalgae. Microalgae have been used over fifty years for the municipal wastewater treatments (Oswald, 1988) and more recently for bioremediation of manure effluents (Mulbry et al., 2008). Manure effluents have a wide range of uses, such as providing liquid or solid digestate via anaerobic digestion of manure in the study field of biogas power plants (Pedrazzi et al., 2015).

In this study, LEDs were chosen as the light source in order to achieve efficient radiative performance with a low cost in terms of both energy saving and device cost. The aim was to compare the combined effect of the chicken manure growth medium and different wavelengths, intensity, density of light per volume of medium(blue, blue combined with red, and white) on the *Neochloris oleoabundans* (Chantanachat and Bold, 1962) growth and to build a prototype of photobioreactor taking into account all these considerations, parameters and variables.

2. MATERIALS AND METHODS

2.1. Microalgal Strain and Growth Medium Conditions

Microalgal species *Neochloris oleoabundans* was obtained from the University of Modena and Reggio Emilia, Department of Life Science (Reggio Emilia, IT). 1 ml of 14 days old microalgae cell suspension with the initial cell concentration of 0.64 x 10^6 cells/ml was transferred into a 250 ml flask containing 100 ml of growth medium (1% v/v). Three different types of mediums were used: BG11, BG11 nitrogen-deprived (BG11-N) and a chicken manure based medium (CM). The BG11-N medium was obtained from the standard BG11 medium recipe without adding $NaNO_3$ as nitrogen source, and corrected for $MgSO_4$.

Commercial dried chicken manure was obtained from the Türem Organic Poultry Company, Ankara. According to the manufacturer it is composed by water soluble Ca 0.25 %, Mg 0.28 % , S 0.32 %, Fe 0.003 %, Mn 0.0056 %, Zn 0.0064 %, Cu 0.0009 %, B 0.0044 %, Mo 0.0005 %, Na 0.26 %, K_2O 3.8 %, total (N + P_2O_5) 7.1 %; total organic matter 62 %, total nitrogen 2.3 %, organic nitrogen 1.9 %, humic + fulvic acid 30.9 %, total organic carbon 19.6 %, total P_2O_5 4.8 %. C/N ratio of the manure is 8.5. Nutrient optimization of the chicken manure was conducted, according to the nutrient content of BG11 which is one of the most common algae growth media. Due to the analysis provided by the manufacturer, suitable amount of dried manure was weighed and diluted in sterile water to obtain a similar nutrient ratio as in BG11 medium. The composition of the nutrient content in the growth medium have been used to calculate the necessary dilution of the chicken manure in sterile water and utilized according to BG11 culture medium components. The pH of each medium was adjusted to 7.3 ±0.1 prior to being autoclaved (121° C, 15 min), and the flasks containing the microalgal cultures were

placed on an orbital shaker at 150 rpm (Heidolph Titramax 101) inside an incubator (Isco SRL-FTD-250 Plus-Cooling Incubator) under 8/16 h photoperiod at 26 °C for a period of 6 days. The flasks were aerated with an air pump. All the experiments were carried out in triplicate.

2.2. Light

The spectrophotometric light absorbance peaks of the microalga *N. oleoabundans* main strain were indicated by measuring the absorption spectrum using UV-Vis/NIR Spectrophotometer (JASCO V-500/V-600 Series Instruments) during the preliminary studies: 436 nm blue wavelength, 665 nm red wavelength (Altunoz, 2016).

The light intensity used in this study was 120 µmol photons m^2s^{-1} (Kahn et al., 1998; Marshall and Hallegraeff, 1999). In order to correctly design the lighting system for each wavelength (blue, red and white) a simple calculation is required. Commercial LED devices exist only in specific power unit sizes (W), different for each colour. The following formula derived from Planck's law has been used to calculate the number of LED devices necessary to achieve the above specified photon flux (120 $\mu mol\ m^2\ s^{-1}$) using devices of different colour by calculating E = energy flux (Wm^2) at each given wavelength [Eq. (A.1)]:

In the case of monochromatic LED such as blue or red, the exact wavelength is given by the producer (Philips, 2012). For white LED which is not monochromatic, the device technical data are calculated by the producer at the normalized wavelength value of 555 nm. This normalization is using the luminosity function, which is an international standard by the Commission Internationale de l'Éclairage (CIE, 1931). It is well known that the determination of energy consumption indicates that blue light has less energy consumption according to white light. A summary of data has been extracted by Philips

technical data sheets, therefore the producer has indicated the average efficiency of the white LED using CIE 1931. Typical efficiency (W/W) and wavelength for different LED light colors are indicated as: 0.39 for red (620-645 nm); 0.29 for red-orange (610-620 nm); 0.15 for green (520-550 nm); 0.26 for Cyan (490-520 nm); 0.35 for blue (460-490 nm); 0.22 for white (555 nm normalized) (Philips, 2012).

In this respect, LEDs were chosen as light source in order to achieve efficient radiative performance with a low cost in terms of both energy saving and device cost. An experimental prototype coverage with LED strips around Erlenmeyer flasks were prepared to provide the proper wavelength to each microalgal culture. The following wavelengths were chosen for the treatments: red (665 nm), blue (436 nm), red and blue combined together in the proportion of 1:3, respectively, and white (6000 K). Each flask with the lights sticked on it was covered with aluminium foil in order to be shaded and protected both from external light and possible interference of other LED wavelengths. In most of the photobioreactor designs, light flux is sent to the culture without dispersions in the environment.

2.3. Cell Concentration, Cell Size, Optical Density and Growth Rate

Concentration of microalgae cells were measured every 2 days by using Improved Neubauer haemocytometer (McAteer and Davis, 1994) under the light microscope, The cell concentration (cells/ml) was calculated according to Guillard (Guillard, 1978).

The cells size was measured by the calibrated software of digital imaging microscope systems (Nikon Corporation Instruments Company, Advanced Research Microscope Eclipse 80i, Japan). The spherical microalgae cells have an approximately similar cell length and width, therefore it is possible to estimate the cell width according to the cell length measured (Sun and Liu, 2003; Verity et al., 1992). To calculate the volumetric

determination, the size of 20 microalgal cells was measured from each culture sample after 5 days of phototrophic growth. Average length of the microalgae cells were compared by using their mean values.

The optical density of *N. oleoabundans* supernatant was measured at 665 nm, which is the maximum red light absorption peak under these experimental conditions. The growth rates were derived from the concentration (Guillard, 1973) on the basis of the following equation [Eq. (A.2)]:

2.4. Photosynthetic Pigment Content

10ml of microalgae was filtered using glass microfiber filters (Whatman, GF/C). Pigments were extracted by re-suspension of microalgae in 90 % methanol for 10 min at 70 °C in a thermal block. Samples were grinded in a mortar and centrifuged at 5,000 rpm for 5 min. Supernatant was taken for measuring the absorbance spectrum between 350-800 nm by the UV-Vis/NIR Spectrophotometer. The analytical determination of chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and carotenoids (C, carotene and xanthophylls) was performed at 470 nm, 652.4 nm and 665.2 nm by subtracting the values of absorbance at 750 nm (Ritchie, 2006). The formula to calculate pigment content was used according to the solvent, which is methanol in this study (Lichtenthaler and Buschmann, 2001).

2.5. Total Lipid Content

A specific volume of culture samples was transferred into falcon tubes and the samples were dried under vacuum concentrator (Eppendorf Concentrator 5301). The microalgal biomass was successively washed twice with 50 mM phosphate buffer (pH 6.5), centrifuged each time for 15 min at 4,000 rpm snap frozen (-80 °C) and lyophilized for 48 h in a Lyophilizer Modulyo (Edwards, Sanborn, NY).

Crude oil was obtained from lyophilized cell material, which was stirred twice each with 500 µl chloroform/methanol (2:1, v/v) at room temperature for 1 h. Solvents were removed from the combined extracts by a stream of nitrogen, and the residual material was dissolved in light petroleum (b.p. 30-40 °C) and applied to a column packed with silica gel 60 (0.063-0.200 mm, Merck) equilibrated with light petroleum. The column was eluted with light petroleum; the total eluate was collected and evaporated until it became dry by a stream of nitrogen to obtain a yellowish crude oil fraction.

2.6. Data Analysis

Within the scope of the data analysis, Friedman and Kruskal-Wallis tests were performed to observe the significance of the experimental results by using Matlab R2016a software (MathWorks, Natick, Massachusetts, USA). The observed data were analyzed to be meaningful according to the tests performed.

3. RESULTS AND DISCUSSION

3.1. Combined Effects of Wavelengths and Growth Medium

In this work, the combined effect of chicken manure medium under three different wavelengths on microalgal cell concentration, optical density, cell size, growth rate, photosynthetic pigment content, total lipid content and their relative correlations were evaluated. Common BG11 medium and the nitrogen deprived BG11 medium were used as control, for a comparison of *N. oleoaboundans* growth behaviour.

The growth conditions in the next sections of this paper will be addressed with the following acronyms: $BG11_W$ (BG11 medium, white light); $BG11_B$ (BG11 medium, blue light); $BG11_{BR}$ (BG11 medium, blue and red light); BG11-N_W (nitrogen deprived BG11 medium, white light); BG11-N_B (nitrogen deprived BG11 medium, blue light);

$BG11\text{-}N_{BR}$ (nitrogen deprived BG11 medium, blue and red light); CM_W (chicken manure medium, white light); CM_B (chicken manure medium, blue light); CM_{BR} (chicken manure medium, blue and red light).

3.2. Cell Concentration

According to the comparison of the three microalgal strains in BG11 medium, $BG11_W$ has the highest value of cell concentration at the end of the experiment (6^{th} day); however, the sharpest slope was observed in $BG11_B$ until the 4^{th} day (Fig. 1a). In the CM medium, the highest concentration is registered at the 4^{th} day for CM_{BR} (14.42% higher than CM_W) which is also the highest value of the whole strains, followed by an abrupt decrease after the 4^{th} day (Fig. 1b). In the BG11-N medium, $BG11\text{-}N_W$ seems to be a good condition for microalgae during the first growth phase, showing higher values of concentration compared to other strains in BG11-N medium; after the 4^{th} day $BG11\text{-}N_B$ has continued to increase contrary to $BG11\text{-}N_W$ and $BG11\text{-}N_{BR}$. However, after the 4^{th} day, $BG11\text{-}N_B$ is the only strain showing an increment compared to the $BG11\text{-}N_W$ and $BG11\text{-}N_{BR}$ (Fig. 1c). As a result, the CM_{BR} condition can be reached at the same cell concentration per ml as in the control ($BG11_W$), but in a cheaper way and in a shorter time. However the highest concentration value does not show the highest biomass, which also depends on the optical density values.

3.3. Optical Density, Growth Rate and Cell Size

After 4 days of microalgae growth, the highest OD values were recorded for $BG11_W$ (Fig. 1d), CM_B (Fig. 1e), and $BG11\text{-}N_{BR}$, respectively (Fig. 1f). At the 4^{th} day, CM_B had the highest OD value with respect to CM_W and CM_{BR} (Fig. 1f).

The highest growth rate is observed for algae grown in CM_{BR} and $BG11\text{-}N_{BR}$ at the 4^{th} day of the experiment (Fig. 1h, i), while CM_W and CM_B decreased since the 2^{nd} day until

the 6th day (Fig. 1h). In the BG11 medium, growth rate appears to be lower than the other mediums in general (Fig. 1g). The CM and BG11-N mediums with combined blue and red light seem to have a better correlation compared to other strains at 4th day (Fig. 1h, i). Meanwhile, growth rate of $BG11_{BR}$ strain reached the highest peak at the 2nd day and then decreased abruptly until the end of the experiment (Fig. 1g).

In the first phase of the growth (4th day), CM_{BR} condition shows the highest cell size value, with the largest cell size increment of 1.411 µm with respect to the other conditions. At the end of the experiment (6th day), the cell size increment of CM_W and CM_B were 0.827 µm and 0.890 µm, respectively, whereas CM_{BR} showed a remarkable increment of 2.294 µm corresponding to the 37% increase in cell size with respect to its initial size within 6 days. BG11-N_W and BG11-N_{BR} also shown significant increments of 1.529 µm and 1.208 µm, respectively, corresponding to 27% and 21% in 6 days.

3.4. Photosynthetic Pigment Content

Pigment content, *Chl a, Chl b, Carotenoid* (carotene and xanthophyll) were measured (Fig. 2). *Chl a* content was observed to start decreasing in $BG11_W$ and $BG11_{BR}$ after the 4th and the 2nd day, respectively, whereas it was observed to be steady for $BG11_B$ during the 6 days' growth (Fig. 2a). In CM mediums under all the light conditions, after 4th day, the *Chl a* content started to decrease, and the maximum peak was observed at the 4th day of all the treatments (Fig. 2b). In the BG11 –N medium, blue and blue combined with red light proved to work well within the 4th day (Fig. 2c). The strains in the same mediums which were exposed to different wavelengths were compared to each other, accordingly white light with BG11 medium ($BG11_W$), blue combined red light with CM and BG11-N mediums (CM_{BR}, BG11-N_{BR}) show the highest values of *Chl b* (Fig. 2d - f). There were no significant differences between the *Chl b* value of CM_W, CM_B, CM_{BR} at the 4th day

(Fig. 2e). In the BG11 medium, all of the strains presented lower values of *Chl b* in comparison to the other mediums in general (Fig. 2d). For the carotenoid content; similarity of $BG11_W$ and CM_B is an important point of this study (Fig. 2g, h), while BG11-N_{BR} presented a higher amount of carotenoid pigment during the early days of the cultivation (Fig. 2i). Generally; for the strains in the CM mediums and BG11-N mediums, carotenoid pigment has higher values in comparison to the BG11 medium.

3.5. Total Lipid Content

During the experiment, total lipid content of the strains were analyzed. According to results, BG11-N_{BR} has shown the highest amount of total lipid as 1.88 mg/g; followed by CM_B, $BG11_W$, CM_W and CM_{BR} which also shown a relatively high amount of lipid content as 1.66 mg/g, 1.61 mg/g, 1,59 mg/g, 1.44 mg/g as described in Figure 3.

Photosynthetic pigment content and lipid content are inversely proportional as depicted in Figure 4. When the amount of lipid content increases, the amount of photosynthetic pigment content decreases, except for CM_B strain. In this strain lipids and photosynthetic pigment contents both shown higher values that is a different behaviour than the other strains.

3.6. Data Analysis

Statistical significance (p) and chi square (χ^2) analysis of cell concentration, cell size and optical density parameters were performed by using Friedman test and resulted in 0.0319, 6.89; 0.0446, 6.22; 0.0183, 8, respectively. The test constrains ($p < 0.05$ and chi square > 5) were fully satisfied. Consequently, Kruskall-Wallis test was performed: chi square and p values are indicated as 29.59 and 1.67941e-06, respectively. According to this result there is a significant relationship between the strains (chi square > 5, $p < 0.05$).

Unicellular green microalgae and higher plants are positively affected by blue light for

protein synthesis or enzymatic activation, while red light seems to influence accumulation of carbohydrates (Senge and Senger, 1991). In the present study, microalgae grown under blue light and blue combined with red light displayed a significant variation in cell concentration regardless of the medium used. In the CM medium, nutrients ratio was calculated to be the same as in the BG11 medium. However, CM medium nutrients were not as bio-available as the ones in the optimized BG11 medium and this could count for the decrease in cell concentration, OD measurement, growth rate, pigment content or lipid content that are usually observed around the 4^{th} day: the nutrients needed for microalgae growth and reproduction have been already taken up, following the 4^{th} day of the microalgae growth renewing the medium or even adding a source of nitrogen would improve growth rate and related parameters of the microalgae cultivation especially for the batch cultures.

Blue light has proven both to enhance the uptake of nitrogen from the growth medium and to inhibit some metabolic activities as well as the uptake of some secondary metabolite as glycine, proline etc (Kamiya and Saitoh, 2002), meanwhile it is related to uptake systems of nitrate, ammonia or urea. In our study, the strains in chicken manure mediums under blue light and blue combined with red light resulted a higher microalgae growth enhancement around 4 - 6 days, which is consistent with faster nitrogen uptake for this strain with respect to the other strains. Accordingly faster use of nitrogen source causes lack of nitrogen and in these culture strains after 4^{th} day optical density, growth rate or pigment content values would have been decreased. The data suggest that adding a nitrogen source should provide a continuous improved growth medium by sustaining the uptake of nitrate under blue light both in chicken manure and BG11-N mediums. According to these results, it is observed that using blue light or blue combined with red

light in chicken manure growth medium is a cost effective way to cultivate a freshwater microalgae culture strain, such as *N. oleoabundans*. It is shown that the maximum specific growth rate of *Nannochloropsis sp.* was under blue LEDs, following this white, green, red LEDs respectively (Das et al., 2011).

The effect of nutrients in each culture medium that has different media formulations can influence the growth dynamics of microalgal cells. According to cell concentration and optical density analysis, the culture strains in the CM medium display a constant cell concentration but also an increasing OD, which means the cell volume of the strains are increasing. Meanwhile, in the microscopic studies, it can be observed that the cell size of the strains in the CM mediums were larger; for instance, CM_{BR} registered an increment of 2.294 µm corresponding to the 37%. It is a significant ratio for the microalgae cell growth with respect to other strains.

As another consequence, while starting a new inoculated subculture system, within the 3^{rd} or 4^{th} day of the growth, microalgae should be exposed to nitrogen starvation to enhance the microalgal growth under blue light or blue combined with red light. After this period, refreshing the medium in case of the CM medium, or adding nitrogen source to the BG11-N medium are ways to enhance the efficiency of the microalgae *N. oleoabundans* culture systems. It is estimated that periodic exposure of continuous culture strains to nitrogen stress would enhance the efficiency of the microalgae culture systems, together with using the blue light, blue combined with red light, and white light which can be replaced with each other periodically, due to the optimal growth conditions of microalgae. The optimum pH for the algal growth has been indicated at 7.5, therefore over 8.5 to 9 the growth is starting to be affected (Richmond, 2004). Total lipid content of microalgae between 25% and 54% lipids (w/w) are common ratios during

nitrogen-depleted growth of *N. oleoabundans* in freshwater conditions (Pruvost et al., 2011; Tornabene et al., 1983).

This study also yielded information about harvesting of the microalgae culture systems. N-limited culture conditions, especially batch cultures obviously appear to be harvested around 4-6 days, related to N content ratio of the growth medium (Deng et al., 2011). According to the amount of total lipid content of the strains, it was an expected result to obtain a higher amount of lipid content in nitrogen deprived growth medium, therefore it is observed that BG11-N_{BR} provided the highest amount of total lipid content which also demonstrates the successful combination of lack of nitrogen and blue combined with red light. The important result in terms of lipid content in this study is that a high amount of total lipid content has been obtained from CM_B which is comparatively higher than $BG11_W$, while it is followed by CM_W and CM_{BR}, respectively (Fig. 3). Early harvest of the cells for lipid production is necessary in N limited cultivation condition. Clearly, manipulation of conditions that combine nutrient deficiency, growth condition and culture duration is important to achieve microalgal cells that allow maximal oil production. In this respect; it is estimated that using chicken manure medium together with blue led light may prove useful to obtain a total oil content from microalgae *N.oleoabundans* in comparison to expensive artificial growth medium together with white light which is a considerable observation of this study.

In CM_B strain both total lipid and photosynthetic pigment content have shown an increase (Fig. 4). This can be interpreted as: the cultivation exposed to blue light and chicken manure medium would have performed mixotrophic growth which involves both heterotrophic and photoautotrophic growths (Stemmler et al., 2016). It has been shown that microalgae growth under mixotrophic conditions provides an increase on the lipid

content over phototrophic and heterotrophic growth (Cheirsilp and Torpee, 2012). Mixotrophic cultures have advantages on providing higher growth rate and biomass yield. Future studies will be focused on this specific behaviour of microalgae *N. oleoabundans*.

In this study, mediums used for microalgal growth resulted that in the BG11-N and CM growth mediums the cell size was larger than BG11 strains on account of the fact that nitrogen starvation leads to larger sized cells (Davis et al., 2012), meanwhile in spite of having larger cells, cell concentration was observed to be lesser compared to other mediums. This data is proven by the correlation of optical density and concentration analysis within the experimental period. While small cell sized strains have a higher number of cells (cell concentration, cell/ml), still optical density value of these strains can result lower than the other strains. This inverse proportion between cell concentration and optical density indicates the importance of cell size or cell biovolumes, or even the length of filamentous microalgae. OD is defined in terms of transmittance which can be measured by using Beer-Lambert Law of Absorbance (Adrien, 1998). A natural microalgae growth, without any stress factor should provide a linear relationship between the cell concentration and optical density value. However in this study, the aim is to adjust the growth parameters to obtain the most convenient growth conditions depending on the required algal product. Accordingly the effects of lights and mediums led to alterations on the microalgal growth which is also related to cell size of the microalgal cells. In this case the cell concentration and optical density relations indicates how to enhance the microalgal culture conditions. The alteration of linear relationship between cell concentration and optical density is due to cell size which is already proven in Section 3.3. As an example, while the cell concentration (cell/ml) of CMw is increasing

(Fig 1b), the optical density value is decreasing (Fig 1e). Therefore, according to Beer-Lambert Law of Absorbance the cell size has to be lower than the other strains within 6th day. This is proven by the lower cell sized CMw strain previously reported in the result section (0.827 μm).

A possible explanation for the enhanced cell size of microalgae grown in CM under blue and blue combined with red light could also concern the medium color. CM is a darker medium compared to BG11, in the process of light diffusion and propagation inside a medium; if the medium of propagation is dark, white light is mostly absorbed by the medium and the cell can get a lower irradiation density. For instance, blue or blue combined with red light irradiated with the same light density is less absorbed by a dark medium than white light leading to higher irradiation density to the cells.

Future studies will be focused on improving the prototype in the field of microscopic power plants, together with the different kind of wastes used as feedstock in the algal cultivation systems.

4. Conclusion

Using chicken manure biowaste as growth medium is a quite cost effective method of microalgae cultivation, which can be enhanced by LED illumination systems. In this study the growth parameters of the model organism *N. oleoabundans* have been monitored: the blue or blue/red LEDs are more efficient on the strains grown in chicken manure medium, while BG11 medium is more efficient with white LED on the microalgal growth. This study performed to adjust the optimum growth parameters according to microalgae species and the necessary product to be obtained from biomass such as secondary metabolite, protein or lipid.

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Captions

Figures

Fig. 1 Effect *of light on: C*ell concentration; Optical density; Growth Rate for 6 day period. Matlab R2014a (MathWorks, Natick, Massachusetts, USA) was used to create the figures.

Fig. 1a In BG11 medium - Cell Concentration

Fig. 1b In CM medium - Cell Concentration

Fig. 1c In BG11–N medium - Cell Concentration

Fig. 1d In BG11 medium - Optical density (665nm)

Fig. 1e In CM medium - Optical density (665nm)

Fig. 1f In BG11–N medium - Optical density (665nm)

Fig. 1g In BG11 medium - Growth Rate

Fig. 1h In CM medium - Growth Rate

Fig. 1i In BG11–N medium - Growth Rate

In each figure, the three lines represent the effect of white light (White), blue light (Blue) and combined blue and red (Blue + Red) on microalgal concentration. **Fig. 1a-c**. Cell concentration - time response of *N.oleoabundans* within 6 days of growth, in different media and under different wavelenghts. **Fig. 1d-f**. O.D. - time response of *N. oleoabundans* within 6 days of growth, in different media and under different wavelenghts. **Fig. 1g-i**. Growth rate – time response of *N.oleoabundans* within 6 days of growth, in different media and under different wavelenghts. All the data reported in the figures are the mean of three biological sample replicas ± SD.

Fig. 2 Effect *of light on: Chl a* content; *Chl b* content; *Carotenoid* (x+c) content for 6 days period. Matlab R2014a (MathWorks, Natick, Massachusetts, USA) was used to create the figures.

Fig. 2a In BG11 medium - *Chlorophyll a* content

Fig. 2b In CM medium - *Chlorophyll a* content

Fig. 2c In BG11–N medium - *Chlorophyll a* content

Fig. 2d In BG11 medium - *Chlorophyll b* content

Fig. 2e In CM medium - *Chlorophyll b* content

Fig. 2f In BG11–N medium - *Chlorophyll b* content

Fig. 2g In BG11 medium - *Carotenoid* content

Fig. 2h In CM medium - *Carotenoid* content

Fig. 2i In BG11–N medium - *Carotenoid* content

In each figure, the three lines represent the effect of white light (White), blue light (Blue) and combined blue and red (Blue + Red) on microalgal concentration. **Fig. 2a-c.** *Chl a* content - time response of *N.oleoabundans* within 6 days of growth, in different media and under different wavelenghts. **Fig. 2d-f.** *Chl b* content. - time response of *N. oleoabundans* within 6 days of growth, in different media and under different wavelenghts. **Fig. 2g-i.** Carotenoid content – time response of *N.oleoabundans* within 6 days of growth, in different media and under different wavelenghts. All the data reported in the figures are the mean of three biological sample replicas ± SD.

Fig. 3 Total amount of lipid content of each strain within 6 days

X-axis represents amount of lipid content (mg/g) within 6 days, Y axis represents sample strains. List of the acronyms are reported in Section 3.1. All the data reported in the figures are the mean of three biological sample replicas ± SD. Microsoft Excel (Redmond, Washington: Microsoft) software was used to create the figure.

Fig. 4 Photosynthetic pigment and total lipid content within 6 days of growth

X-axis represents amount of total lipid and total pigment content (mg/g) within 6 days' Y axis represents sample strains. List of the acronyms are reported in Section 3.1. All the

data reported in the figures are the mean of three biological sample replicas ± SD. Microsoft Excel (Redmond, Washington: Microsoft) software was used to create the figure.

Figures

Fig. 1 Effect *of light on: C*ell concentration; Optical density; Growth Rate for 6 day

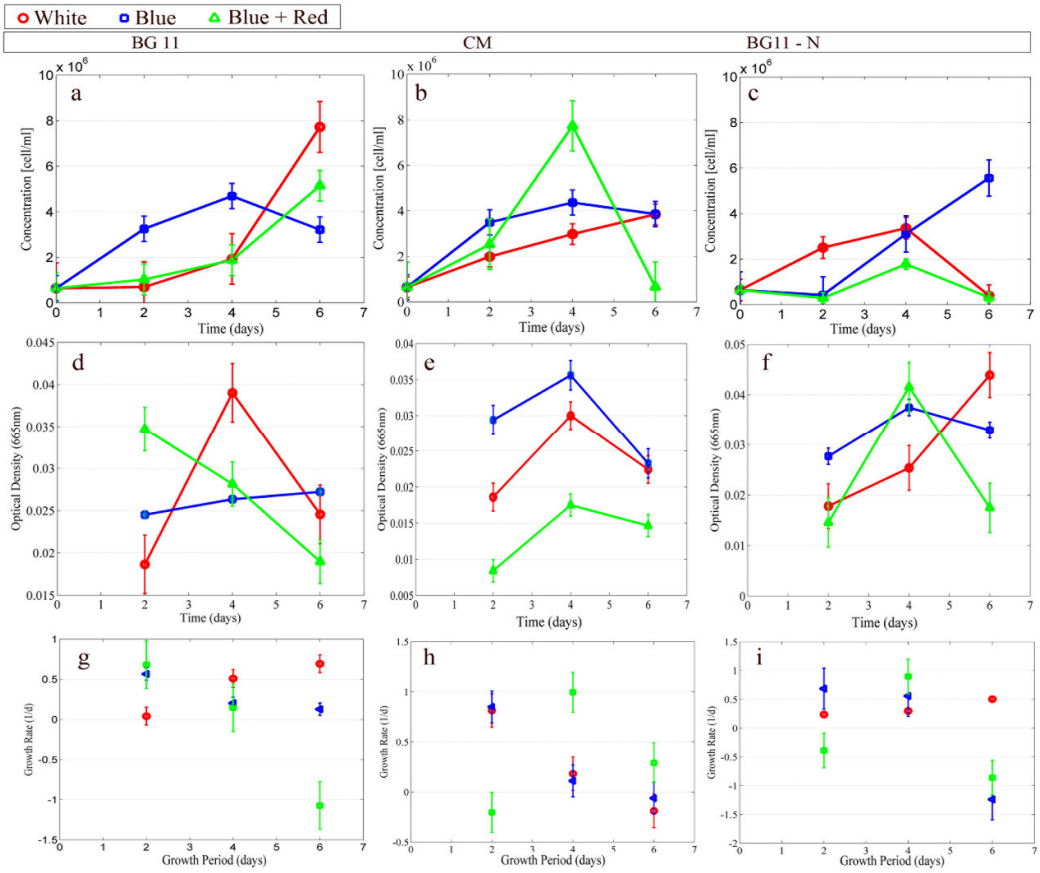


Fig. 2 Effect *of light on: Chl a* content; *Chl b* content; *Carotenoid* (x+c) content for 6 days period

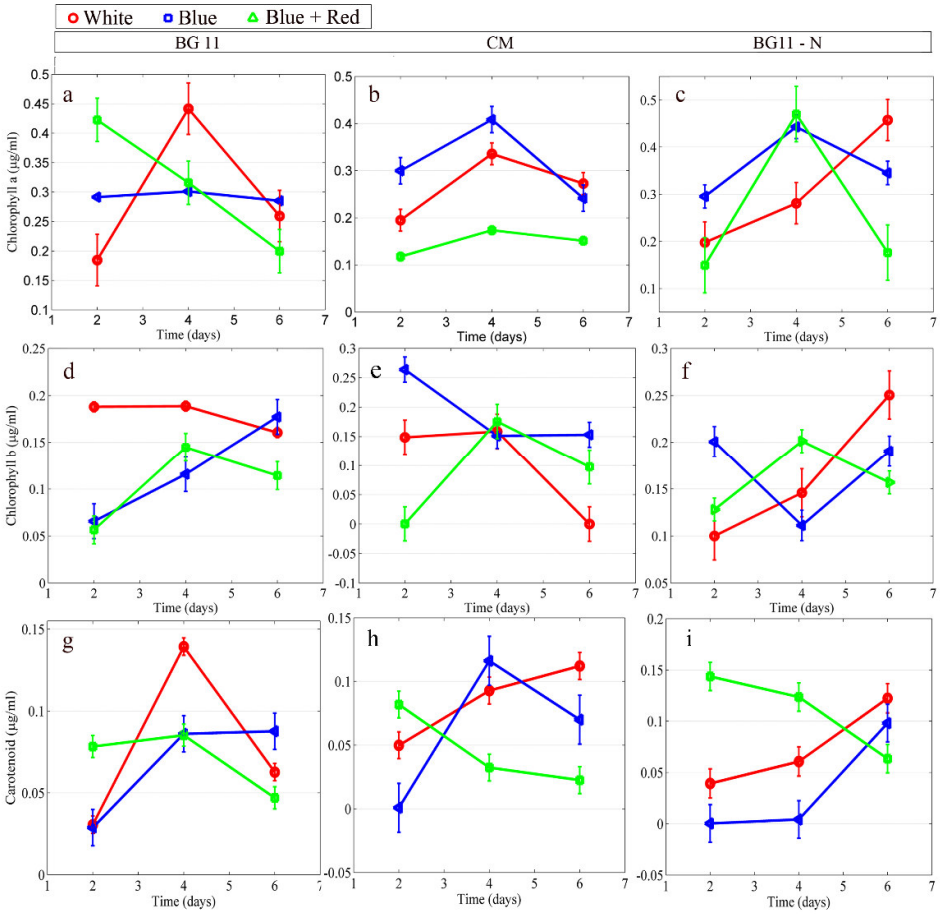


Fig. 3 Total amount of lipid content of each strain within 6 days

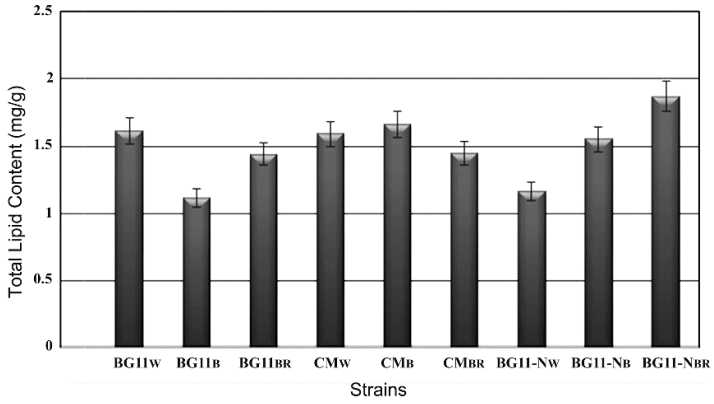
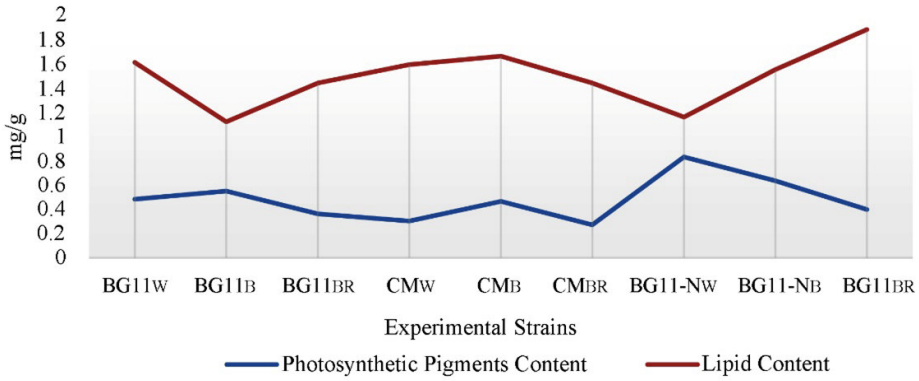


Fig. 4 Photosynthetic pigment and total lipid content within 6 days of growth



APPENDIX

Eq. (A.1): Conversion of photon flux (µmol m^2 s^{-1}) to energy flux (Wm^2) to evaluate the energy irradiated at each wavelength:

$$E = (n \times c \times h)/\lambda$$

where n is the photon flux, c is the speed of light, A is Avogadro's number, h is the Planck's constant, and λ is the given wavelength [m].

Eq. (A. 2): Growth Rate

$$\mu = [(\ln(Wi) - \ln(Wf)]/\Delta t$$

where μ is growth rate (t^{-1}), W_i is initial cell concentration (cell/ml); W_f is final cell concentration (cell/ml); Δt is length of the time interval.

HIGHLIGHTS

- Chicken manure was used as microalgal growth medium for *Neochloris oleoabundans*.
- Different wavelengths of LED such as white, blue and blue-red combined were tested.
- The basic design of a chicken-manure-fed photobioreactor is defined.