LIGHT INTENSITY AND PHOTO PERIOD EFFECT ON GROWTH AND LIPID ACCUMULATION OF MICROALGAE CHLORELLA VULGARIS AND NANNOCHLOROPSIS SP FOR BIODIESEL PRODUCTION

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Abstract- The application of microalgal oil for energy purposes continues to present a number of challenges, including the optimization of culture conditions. In this study, the two light intensities at 2.5 Klux and 5 Klux were used in light-dark cycles of 8:16, 16:8, 0:24 and 24:0 hour to investigate the effect of light intensity and photo period on biomass production and lipid productivities in microalgae, Chlorella vulgaris and Nannochloropsis sp. in view of optimizing the illumination level for biodiesel production through algal oil. Chlorella vulgaris and Nannochloropsis sp were cultured in Guillard and Rither`s f/2 media for 21 days and 14 days growth periods respectively in a laboratory scale closed experimental set-up. The cultures were aerated with mechanical aerators. Finally, cells were harvested by flocculation and dry weight and the percentage lipid content were estimated. Significantly higher average dry matter yield was observed when the cells cultured under 5 Klux light intensity than the 2.5 Klux during all the tested photo periods in both species. In the present study, it was found that the significantly highest dry biomass of 0.63 g/L was recorded with light-dark cycle of 16:8 hour while highest percentage lipid content of 13.9 % was obtained light-dark cycle of 8:16 for C. vulgaris at 5 Klux light intensity. In Nannochloropsis sp, light-dark cycle of 16:8 hour recorded the significantly highest dry biomass of 0.62 g/L while 16:8 and 12:12 hour light-dark cycle reported the significantly highest percentage lipid content of 12.36 % and 12.96 % respectively. However, significantly higher final lipid yield of 0.07 g/L and 0.08 g/L was observed in 16:8 hour light-dark cycle for both C. vulgaris and Nannochloropsis sp respectively with 5 Klux light intensity. Therefore, it can be concluded that, the amount of light intensity affect significantly to the lipid yield of micro algae and the optimized photo period for culturing Chlorella vulgaris and Nannochloropsis sp. for lipid production, is to cultivate them with a light-dark cycle of 16:8 hour.

Index Terms- Microalgae, lipid yield, light intensity, photo period

I. INTRODUCTION

Fossil fuel as the major source of energy has many negative environmental impacts, such as problems with global warming and emission of greenhouse gases. Further, increasing fuel demand worldwide also increases the cost of petroleum fuels [9]. Therefore, a variety of biomasses from different sources, including forestry, agricultural, and aquatic sources have been investigated as the feedstock for the production of different biofuels like biodiesel, bio-ethanol, bio-hydrogen, bio-oil, and bio-gas [3]. However, producing biodiesel from vegetable crops is time consuming and requires great areas of arable land that would compete with the one used for food crops, leading to starvation in developing countries [14].

Feedstock lipids generated from phototrophic microorganisms, mainly microalgae have higher areal yields than plants and offer many potential advantages as a non-food feedstock for biodiesel production. Further, they are one of the fastest growing photosynthesizing organisms which can complete an entire growing cycle every few days [10]- [13]. Some other advantages of microalgae-derived biodiesel are: higher production rates per unit area compared to land grown crops, the possibility to grow on marginal or non-arable land, utilization of variety of water sources (seawater, waste water, fresh water), both CO_2 neutral fuel manufacture and CO_2 sequestration, non-toxic, biodegradable and renewable fuel [11].

As with all plants, microalgae reproduce themselves using photosynthesis. They require only inorganic compounds such as CO_2 to convert light energy into chemical energy (stored in chemical bonds of produced compounds, such as lipids) [3].

Microalgae are traditionally considered as a primary food source for larval and juvenile bivalves and for the larvae of some crustaceans and fish species in aquaculture. Several species of microalgae have been shown to produce high-value products such as protein, polyunsaturated fatty acid, antioxidants, vitamins, bioactive compounds and natural colorants [8]. Some microalgal species, including some Chlorella sp, Nannochloropsiss sp, Dunaliella sp [2] and Scenedesmus sp [4] have been reported to accumulate large quantities of lipids up to 50% by weight of dry biomass. These lipids can be converted into biodiesel by a chemical process called transesterification and this biodiesel can be used directly or as a blend with diesel fuel for diesel engines [4]. However, the growth characteristics and lipid accumulation of microalgae are known to significantly depend on the cultivation conditions. According to previous studies the lipid content in some microalgae could be increased by changing the nutrient concentration, temperature, CO_2 aeration

fixation, salinity, light intensity and photo period [5]-[8]- [16].

As light is the source of energy which drives photosynthesis of microalgae; light intensity, spectral quality and photoperiod plays an important role in enhancing the biomass productivity of microalgae. Light may be natural or supplied by fluorescent bulbs. Too high light intensity (e.g. direct sun light, small container close to artificial light) may result in photo-inhibition and photooxidation while low light levels will become growth-limiting. Also, overheating due to both natural and artificial illumination should be avoided [12]. Light intensity should be delivered evenly over the illuminating surface of culture vessel and with adequate amount of photosynthetically active radiation (PAR) to enable photons reach the cell in culture [15]. Due to this, the light intensity at which culture growth becomes saturated is an important factor in determining the light utilization efficiency. Further, microalgae needs a light/dark regime for productive photosynthesis, it needs light period for a photochemical phase to produce Adenosine triphosphate (ATP), Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and also needs dark for biochemical phase synthesize essential molecules for growth. In this regard, cellular content of protein, carbohydrates and lipids in microalgae can be varied through variation of photoperiod [1]. Therefore, the light effect on microalgal growth should be studied to evaluate the requirements of artificial light for their cultures.

There are many factors that should be considered simultaneously in the selection of the most suitable species or strains for biodiesel production. Nannochloropsis sp is a marine microalgae sp belonging to the class of Eustigmatophyceae. The genus is widely used in aquaculture due to its relatively high growth rate, resistance to mixing and contamination together with high nutritional values and high lipid content. These characteristics also fit the needs of the biofuel industry [11]. On the other hand, Chlorella vulgaris is a promising freshwater non-motile unicellular alga, which can be grown in many conditions around the world; they can serve as an example of phototrophic, heterotrophic and mixotrophic growths [7].

In this regard, the present study focuses on determining the effect of light intensity and photoperiod on growth and lipid yield of two algae species viz., Chlorella vulgaris and Nannochloropsis sp in laboratory scale in view of its possible utilization as a raw material for biodiesel production.

II. MATERIALS AND METHODS

Microalga Nannochloropsis sp was grown in 35 ppt (parts per thousand) saline water while Chlorella vulgaris was cultured in freshwater (zero salinity) for 14 days and 21 days growth periods respectively. The two light intensities at 2.5 Klux and 5.0 Klux were used in light-dark cycles of 8:16, 16:8, 0:24 and 24:0 hour to investigate the effect of light intensity and photo period on biomass production and lipid productivities in both species in a laboratory scale closed experimental set-up. Inside the growth set-up, light was provided by fluorescent bulbs (40 W) which were fixed at a distance of 25 cm from the cultures. Temperature inside the growth set-up was between 28 – 32 °C during the experimental period.

All the cultures were provided with aeration and the medium used for cultivation was Guillard and Ryther's modified F/2 media [6]. All the glassware and media were sterilized prior to inoculation in an autoclave for 20 min at 121°C in order to prevent any contamination during the early stages of growth.

A. Dry weight and lipid yield analysis

The experiment was terminated on the stationary phase of the growth cycle. The biomass was harvested by chemical flocculation method using Na0H as the chemical agent. The pellet was washed three times with distilled water dried in an oven at 105˚C, for 6 hours and weighted for the dry weight analysis. The lipid content of the harvested algae samples were determined using Soxlet apparatus. Finally lipid yield was calculated as the lipid content multiplied by the biomass yield.

B. Statistical analysis

Average values of the dry biomass weight and the percentage lipid content of three replications and their standard deviations were calculated. One-way Analysis of Variance (one-way ANOVA) was performed at 95 % level of probability in order to test the significance differences. When the test resulted $p < 0.05$ a Turkey post-hoc test was performed for pair wise comparisons. Since the lipid yield was calculated as the average value of lipid content multiplied by the average biomass concentration, it is reported as a single value without standard deviation.

III. RESULTS AND DISCUSSION

Microalgae are photosynthetic organisms, i.e., they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this process. Photosynthetic process of microalgae includes light reactions as well as dark reactions. Light reactions occur only when cells are illuminated. Dark reactions also known as carbon-fixation reactions occur both in the presence and absence of light [2]. In overall, the cells transform light energy into chemical energy, which is stored in high-energy compounds such as ATP and NADPH. These compounds were later used in the dark phase carbon-fixation reactions to synthesize metabolic molecules essential for growth [8]. In this regard, considering light as the most important energy source for the photoautotrophic algae, current study focused on the effect of light intensity and photo period on

growth and lipid accumulation of two promising microalgae species for biodiesel production.

As shown in the Fig. 01 and 02, in both species dry biomass weight was significantly higher in treatments with higher light intensity (5 Klux) than the treatments with lower light intensity (2.5 Klux) in all the photo periods. Significantly highest dry biomass weight of 0.63 g/L and 0.62 g/L was reported for Chlorella vulgaris and Nannochloropsis sp respectively when cells cultured in 5 Klux light intensity in 16:8 hour light dark period.

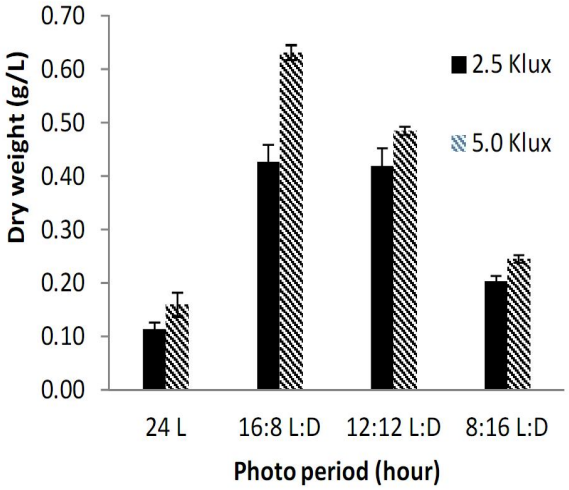


Figure 01: Dry weight of C. vulgaris under different light intensities and photo periods

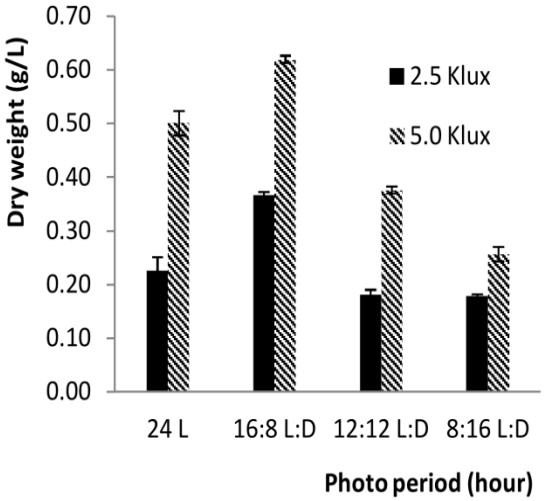


Figure 02: Dry weight of Nannocholoropsis sp under different light intensities and photo

This result is in agreement with previous studies. Reference [2] reported higher dry biomass productivity of 0.271 g/L/ day when using 6 Klux light intensity than the results obtained with 4 Klux and 7 Klux light intensities for C. vulgaris. According to the literature, the amount of light received and stored by the cells has a direct relationship with the carbon-fixation capacity, consequently determining the biomass productivity and cell growth rate. Cells grown under saturated light conditions accumulate carbohydrate and triacyglycerals as storage materials, resulting in high content of biomass. However, at very high irradiance, photoinhibition may lead to cell damage thus inhibiting the growth rate and finally causes cell death [2]. Hence, an adequate supply of light energy is most critical parameter in algal growth and lipid productivity. The optimum level would depend on the microalgae`s photosynthetic ability to fully utilize the photo-energy.

Other than varying light intensity, the experimental setup also takes into account the light/dark cycle variation. As mentioned earlier, photo-period affects microalgal lipid and biomass production. In the present study, it was found that the significantly highest dry biomass of 0.63 g/L was recorded with light-dark cycle of 16:8 hour while highest percentage lipid content of 13.9 % was obtained light-dark cycle of 8:16 for C. vulgaris at 5 Klux light intensity (Fig. 03). In Nannochloropsis sp, light-dark cycle of 16:8 hour recorded the significantly highest dry biomass of 0.62 g/L while 16:8 and 12:12 hour light-dark cycle reported the significantly highest percentage lipid content of 12.36 % and 12.96 % respectively at 5 Klux light intensity (Fig. 04). Reference [2] also found that 10:12 h and 12:12 h (light: dark) as the optimum photoperiods for C.vulgaris and Nannochloropsis oculata respectively. Scenedesmus sp. cultured under mixotrophic condition illumination with fluorescent lamp (10.8 µmoles.$m^{-2}s^{-1}$) with a light-dark cycle of 16:8 h has produced the highest number of cells and also the the highest crude lipid percentage of 11.2 % [4].

Apparently this is because during the light period (24:0-hr cycle), microalgae perform photo reduction which absorbs energy from light and stores it in energy-carrying molecules such as ATP and NADPH through exergonic reactions. These energy-pool molecules can be used for the synthesis of biomolecules that promote the growth of the microalgae [8]. Under 16:8, 8:16 and 12:12 hour cycle, the alga has a dark period in which light-independent reactions can be performed via the Calvin cycle which also operates during the dark phase of photosynthesis. These chemical reactions convert carbon dioxide and other compounds into glucose by using ATP and NADPH from the photo reduction. Microalgae can oxidise supplemented glucose for energy and then store some excess energy in the form of lipids [3]. Hence, the ability of alga to exhibit high lipid productivity depends on its capacity to store energy during light period and utilize it during dark periods.

Reference [2] reported that at low light intensities, lipid productivity will also be low due to limitation of the energy required for the sequence of photosynthetic reactions. Similarly, in the present study, percentage lipid content was significantly

higher in larger light intensity (5 Klux) than lower light intensity in Chlorella vulgaris for all the photo periods (Figure 03). But the result for Nannochloropsis sp was somewhat different than these. When providing light for all 24 hours, percentage lipid content of Nannochloropsis sp was higher at 2.5 Klux than 5 Klux light intensity. The reason behind this would be exposure to high light intensity all the time (24 hour) that may damage the cells thus inhibiting the growth rate [8].

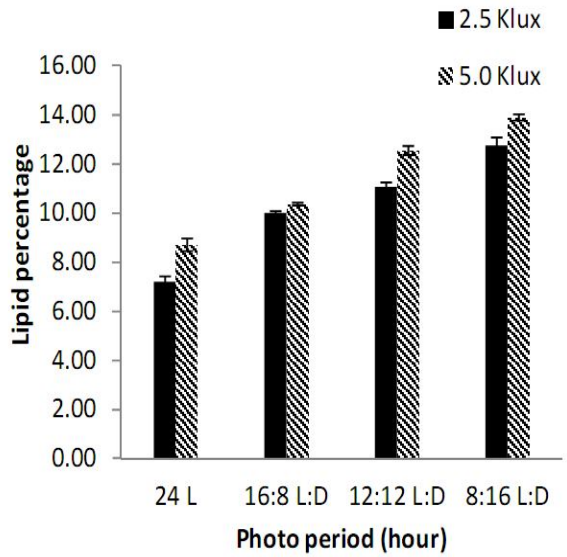


Figure 03: Percentage lipid content of C. vulgaris under different light intensities and photo periods

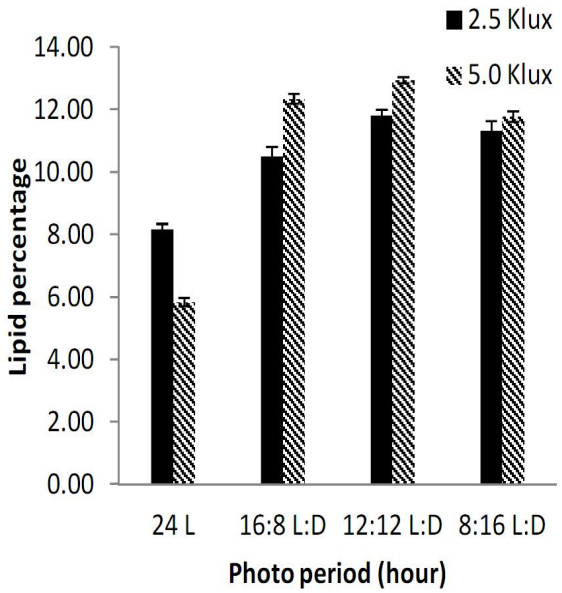


Figure 04: Percentage lipid content of Nannocholoropsis sp under different light intensities and photo periods

However the final lipid yield which is the multiplication of dry biomass by percentage lipid content was significantly highest in 16:8 hour light-dark cycle for both species with 5 Klux light intensity. For C. vulgaris highest lipid yield was 0.07 g/L while Nannochloropsis sp 0.08 g/L (Fig. 05 and 06).

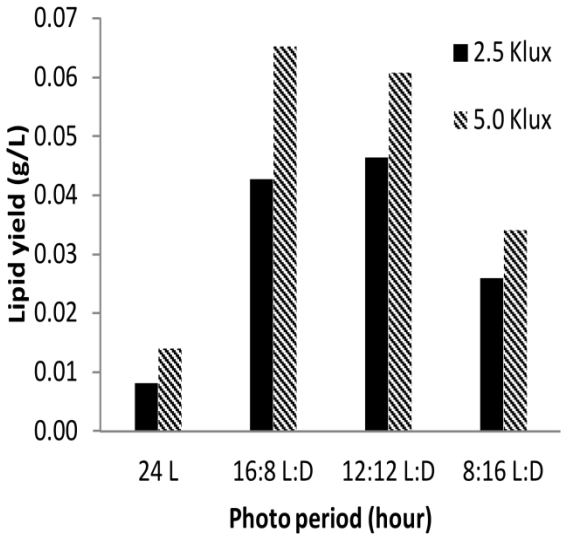


Figure 05: Lipid yield of C. vulgaris under different light intensities and photo periods

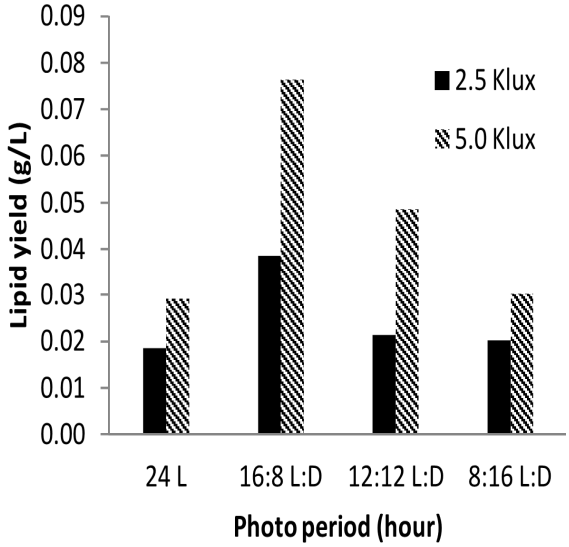


Figure 06: Lipid yield of Nannocholoropsis sp under different light intensities and photo periods

CONCLUSION

The optimised light condition for culturing Chlorella vulgaris and Nannochloropsis sp. for lipid production, is to cultivate them with a light-dark cycle of 16:8 hour. Amount of light intensity also affect significantly to the dry biomass weight, percentage lipid content and lipid yield. In the present study using 5 Kulx light intensity reported highest lipid yield than 2.5 Klux. However, provision of artificial light is an expensive energy source so the algal biomass and lipid product are still expensive to be used as raw materials for lipid production. Further studies should focus on decreasing the cost by using natural light sources.

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