Tarım Makinaları Bilimi Dergisi (Journal of Agricultural Machinery Science)
2011, 7 (4), 355-360

Biodiesel Potential of Chlorella Kessleri Grown under LED and Fluorescent Illumination Sources

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Received (Geliş Tarihi): 08.05.2011 Accepted (Kabul Tarihi): 09.07.2011

Abstract: Many species of microalgae produce lipids/oils that can be extracted for biofuel production. High oil-yielding microalgae can be produced in open ponds or closed photobioreactors. Illumination sources should be chosen carefully because illumination cost is the major cost in microalgae production in closed systems. In this research Chlorella kessleri, one of the highest oil producing microalgae producing 18-58% oil on a dry basis, was grown in a closed photobioreactor. Three illumination sources of Blue LED, Red LED and Fluorescent lamps were used separately. The algae grown under these illumination sources were evaluated for the cell count, cell weight, cell size and lipid content and their biodiesel production potential were assessed. The results showed that fluorescent light was the most efficient illumination source producing the highest number of cells ($3.5x10^6$ cell/mL) and red LED light was the most effective illumination source producing the highest weight (2.7g/L).
Key words: Microalgae, LED, photobioreactor, Chlorella kessleri, biomass, algae, PBR, cell weight, cell count, cell size

INTRODUCTION

The transportation and energy sector are mainly responsible for the greenhouse gas (GHG) emissions. The agriculture sector is the third anthropogenic sector responsible for gas emission, in which the most important gases are nitrous oxide (N_2O) and methane (CH_4) (Anonymous, 2004). Due to society's dependence on fossil fuels, which will be diminished in the near future, scientists have been investigating new, clean and reliable energy sources all over the world for years. Currently the renewable energy options include solar energy, wind, hydroelectric, geothermal and biofuel (Dewulf and Langenhove 2006; Gilbert and Perl 2008). Biodiesel and bioethanol are the most common biofuels which can be used in combustion engines with little or no modifications of the engine fuel systems. Biofuels as alternative sources of energy, are more expensive than fossil fuels. Biodiesel is produced from vegetable oils which is a food source. Because of this situation, vegetable oil prices increase and prevent its usage even if it has several environmental advantages compared to petroleum diesel.

Microalgae have emerged as an alternative energy source. Microalgae can be used for both biofuel production and for wastewater treatment. Microalgae have the ability to reduce greenhouse gas emissions through consuming CO_2. Although microalgae was reported as an alternative energy source in several articles, its production is still not competitive on a cost basis with petroleum diesel (Kanel and Guelcher, 1999; Bijl et al., 2004; Yokochi et al, 2003). In the long term, as crude oil sources diminish and oil prices increase, alternative energy sources will have to become available in an urgen fashion. Biodiesel potential of microalgae as an alternative energy sources is important.

Microalgae can be produced in open or closed Photobioreactors (PBR). The most important factor for microalgae production is illumination. Production of high density micro-algal cultures in open reactors are limited by the sun being the light source, evaporation losses, the release of unabsorbed CO_2 into the atmosphere and pollution (Lee, 2001). The ideal environment for the production of high density

microalgae is a closed Photobioreactor (Pulz, 2001; Tredici and Zitelli, 1997). The major obstacle for commercial use of closed PBRs is the high operating costs which is generally associated with illumination (Lee and Palsson, 1994; Barta et al, 1990, Richmond, 2004). For widespread commercial use, photobioreactors are required to be relatively cheap, durable and reliable and have an efficient light source. LED light sources are suitable for use in Photobioreactors. LED lights have advantages, such as high luminous efficiency, low energy consumption, and long life span over other light sources (Koc et al., 2009).

Many kinds of microalgae species have substantial quantities of oil which can contribute to high oil yield. High oil content producing microalgae species, such as Chlorella kessleri, Botryococcus braunii and Syneehococcus sp have become prominent in recent years (Borowitzka, 1992; Chisti, 2007). Average oil content of microalgae varies between 1 and 70% (db) under certain conditions (Mata et al. 2010). Oil content of Chlorella kessleri is between 18 and 57 % (db) and has a good potential as a biodiesel feedstock.

Every microalgae species has its own their own fatty acid composition which helps to determine their biodiesel potential. Microalgae cell structures are composed of saturated and unsaturated fatty acids with 12-22 carbon atoms, some of them are within the ω3 and ω6 families. In this study all environmental conditions, except for the light sources and light intensities, were kept constant when growing Chlorella kessleri (UTEX 398), microalgae. In the experiments, red and blue LEDs and fluorescent lights were used as illumination sources. The results after a period of eight days of growth used to determine the potential of Chlorella kessleri for biodiesel production.

MATERIALS and METHOD

Three Photobioreactors made from Plexiglas were used for the experiments. To mix the microalgae and growth medium in the PBR, tubes placed at the bottom of the photobioreactors were used to inject air into the PBR. The air passing through the tubes was from an air compressor. CO_2 (95% CO_2 and 5% nitrogen) was added to the air as needed. Flow meters were used to measure and control the Carbon dioxide and air flows (Cole Palmer Inc). For illumination, 270 x 270mm (10.67 x 10.67 inch) LED panels were obtained from DAKTRONICS (Brookings, SD), which had 59 blue LED and 384 red LED lights. The experiments were conducted by supplying constant current (maximum 2.6 A) for each LED panels (Figure 1).

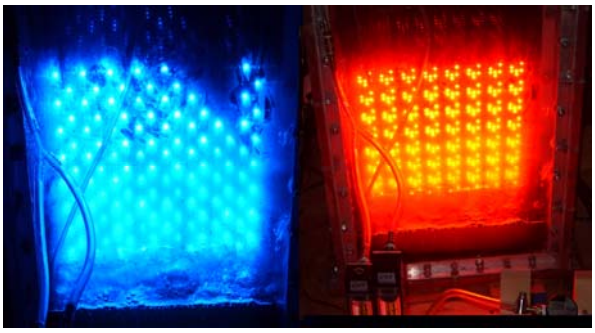

Figure 1. LED panels

For measuring the wavelength and intensity of the light sources, a three-channel fiber optic spectrometer (Avantes Inc.) was used. The features of the illumination sources are given Table 1.

Light (or radiance) is the main source of energy for photosynthetic algae to produce food using the photosynthetic process. Light propagates both as waves and discrete packets called photons. Each photon consist of a discrete quantity of energy. Microalgae are very efficient at absorbing light energy. But light sources should be positioned at a proper distance. Increasing the intensity of light to increase its penetration depth in PBR can lead to micro damage of the algae from overheating or photo-oxidation (Lee, 1999). In these experiments light sources were adjusted to about 2 cm away from the PBR walls.

Mixing is one of the most important factors to obtain high microalgae cell concentrations density of microalgae within the growth medium in photobioreactors.

Table 1. Features of illumination sources

	Number of Lamps	Voltage (V)	Current (A)	Power Input (W)	Wavelength (nm)	Light Intensity ($\mu Wcm^{-2}nm^{-1}$)	Intensity (lux)	Energy of Photon (J)	PAR ($\mu mol.m^{-2}.s^{-1}$)
Blue LED panel	58	6.5	2.6	16.9	467	557	3019	$4.25x10^{-19}$	217
Red LED panel	384	11	2.6	28.6	659	272	1389	$3.01x10^{-19}$	123
Fluorescent	1	110	-	40	-	163	2936	$3.97x10^{-19}$	71

Algae cells can not benefit from the light sources and nutrients without effective mixing. In addition, mixing can prevent algae from sinking to the bottom in the PBR. In the experiments, mixing was accomplished with air supplied by a 5.5 HP compressor (Coleman Power, U.S.). The air was sent through tubes with a porous membrane (PENN PLAX) into the PBR. The tubes were placed at the bottom of Photobioreactors so that air bubbles rose up through the PBR escaping from the top of the PBRs. The size and quantity of air bubbles were established by adjusting the air flow rate which also adjusted the membrane opening size. Air flow rate was measured with a flow meter.

Culture Medium

Growth medium for microalgae production was obtained from UTEX (The Culture Collection of Algae at the University of Texas at Austin). UTEX N-8 growth medium for Chlorella kessleri (UTEX 398) was used. A culture medium of 9 L was prepared in a batch with distilled water for microalgae and 3L culture medium was used for each of the PBRs placed under different illumination sources. In order to avoid bacterial contamination, the water was treated by a RO/DI device (Thermo Fisher Scientific Inc.) which uses ultraviolet light to destroy bacteria in the water. The initial cell concentrations for the experiments were 1.1 x 10^6 cells /ml for Chlorella kessleri (UTEX 398). The same concentrations were used for each illumination source (Blue, Red, and Fluorescent).

Data collection

During the experiments, environmental conditions such as pH, temperature, ORP, and gaseous CO2were monitored using sensors (Cole-Parmer Instrument Company). All the data obtained from the sensors was stored on a computer via a data acquisition system. A program was developed in the G-programming code of Labview 8.5 software (National Instruments). In addition, the sensors were power supply set to provide a constant 13V.

Cell Analysis

Cell analysis was performed with a microscope and hemacytometer. Samples of 10 ml were taken from the PBR and coated with 1 ml of isotonic diluents (Fisher Scientific), which increased the visibility of the microalgae for microscobic observations. Later three-1 ml samples were mixed and counted. A microscope (Electron Microscopy Sciences) was used to count the microalgae cells. A picture was taken by a digital camera mounted on the microscope. The captured image was transferred to a computer with the aid of Infinity 2 software. The measurements were repeated every 24 hours. Three samples were taken each time and the average cell concentrations was recorded. The number of cells in an area of 0.01 mm^2 on the hemacytometer was multiplied by 10^4 to calculate the total number of cells per 1 ml.

To measure biomass weight, filter paper with a 10 µm pore size was used. The filter paper was weighed before the algae solution was placed on it. A 10 ml sample was placed on the filter paper and water was drained. Then the filter paper was placed in a vacuum drying oven (Cole-Parmer) and dried for 5 hours at 75 °C. The filter paper with dried algal biomass was reweighed with a precision balance. The weight of filter paper was subtracted from the algae/filter paper weight to determine the weight of the microalgae in the sample. The results were recorded.

Biomass composition

The dried biomass samples were analyzed for oil content. The oil content of the samples was determined by using a Soxhlet extractor. Hexane was

used as the solvent to extract the oil from microalgae samples. The extraction continued for 8 hours at 70 °C. After the extraction, hexane was separated from oil via distillation. The extracted oil sample was then analyzed for fatty acid content using a gas chromatograph GC.

Measurement of Fatty Acid Composition

A Varian 3400 equipped with Varian 8200 auto sampler and a FID detector was used to determine the fatty acid composition of the Black Seed oil. A 30 M x 0.25 mm DB-WAXeter fused silica column (Agilent Technologies) was used for the measurement. Oil samples were quantitatively weighed in the volumetric flask to prepare a solution of approximately 5-6 mg hexane. A known aliquot containing approximately 4-5 mg of oil was pipetted to a reaction vial. One milliliter of internal standard (C17:0 methyl ester) in hexane was added and mixed well. The hexane was evaporated to dryness using a stream of nitrogen. Two ml of BF3/Methanol reagent (Supelco) was added, mixed and capped tightly. The reaction mixture was heated to 100 °C for 30 min with occasional shaking. The mixture was cooled and 1 ml of deionized water was added. The methyl esters of fatty acids were extracted with 2 ml of hexane. The extract was dried with anhydrous sodium sulfate and 3 µl were injected to Gas Chromatography. Quantitative analysis was carried out using standard fatty acid methyl esters and C17:0 (methyl ester) as internal standard. Results of the analysis were represented as percent fatty acid in the oil samples. Helium, at a rate of 1 ml/min, was used as a carrier gas, injector temperature was set at 250 °C and the column temperature was programmed to increase the temperature from 170 °C to 225 °C at a rate of 1 °C/min.

RESULTS and DISCUSSION

All results evaluated in this article are due to the effect of illumination sources on biomass dry weight (DW) and cell count yield of the Chlorella kessleri. For the experiments, temperature was kept around 23 °C and the intensity of illumination sources was stabilized at 2.6 mA. Growth medium pH value was initially kept at 6.02 for Chlorella kessleri. If the growth medium became more basic, CO_2 was added to the photobioreactor. Figure 2 shows the pH for 8 days. Medium pH increased throughout the experiment. During 8 days of experiments, pH ranged from 6.0 to 7.2 for Chlorella kessleri. The rate of increase on the pH values were highest during the first 3 days and pH values reached a maximum at the 4th and 5th days under red and fluorescent illumination sources. Atmospheric carbon dioxide was used as the carbon dioxide source until 4th day. After the 4th day CO_2 was added to the PBRs. Addition of CO_2 reduced the pH during the rest of the experiments. Increasing pH indicated that carbon dioxide was being consumed by the algae faster than it was supplied or possibly the algae were producing a metabolite that tended to be basic. In treatments, the highest pH was measured in PBRs illuminated with red LED and fluorescent light sources indicating that the algae in these two PBRs were consuming more carbon dioxide thereby growing in number of cells or size faster.

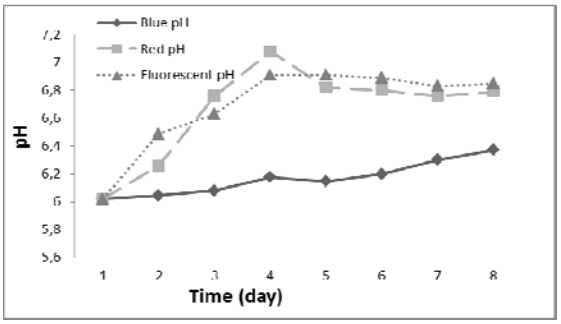

Figure 2. Evaluation of pH change with time.

The highest increase in cell concentration was obtained under fluorescent light illumination ($3.5x10^6$ cell/mL). Cell concentrations increased during the experiments under fluorescent and red illumination sources. Whereas, there wasn't any increase in cell concentration under blue light (Figure 3).

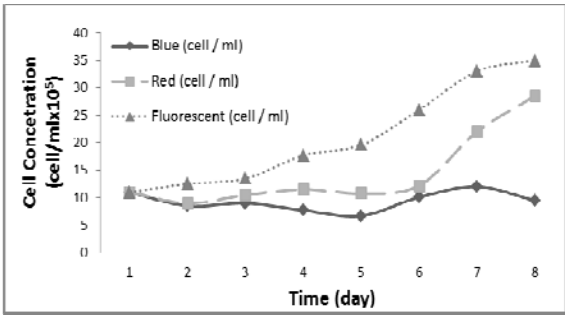

Figure 3. Evaluation of cell concentration change with time.

Cell mass is an important parameter showing the biomass potential of the algae. The measured values related to the cell mass concentration are shown in Figure 4. Red LED light showed the highest increase in cell mass concentration. The highest cell weight (2.7g/L) obtained on the 7th day under the red illumination. Fluorescent light also affected the cell weight positively. Cell weight continued to increase from the first day until the end. Blue light didn't show any effect on the cell weight and cell weight decreased under blue illumination source.

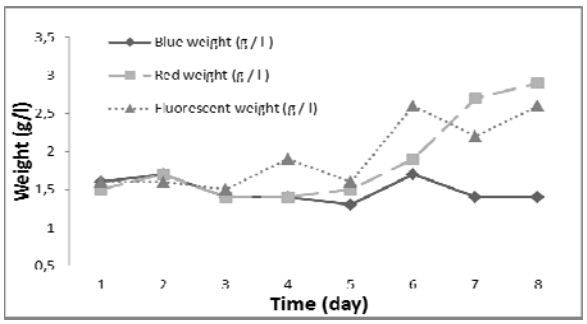

Figure 4. Evaluation of cell mass change with time.

For photosynthesis, CO_2 is very important parameter for microalgae cultivation. CO_2 was added to PBRs based on the pH value. The concentrations of CO_2 from the experiments are shown in Figure 5. The rate of CO_2 gas exchange was quite high under all illumination sources. CO_2 concentration in the exhaust air from the PBRs ranged from 1200 to 2300 ppm. The largest CO_2 concentration was found in the PBR under blue illumination source (over 2226 ppm on the seventh day). In the PBRs, the growth rate for all species of algae grown under blue light have remained very low. Under blue light more carbon dioxide was passing through the PBR and not being consumed by the algae resulting in a lower pH (Figure 2). In addition, dissolved oxygen rate was very low under blue illumination source (Figure 6).

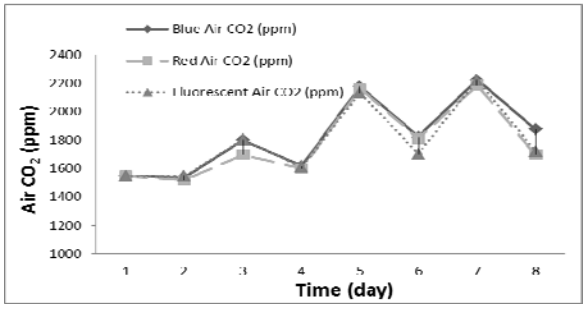

Figure 5. Evaluation of CO_2 change with time.

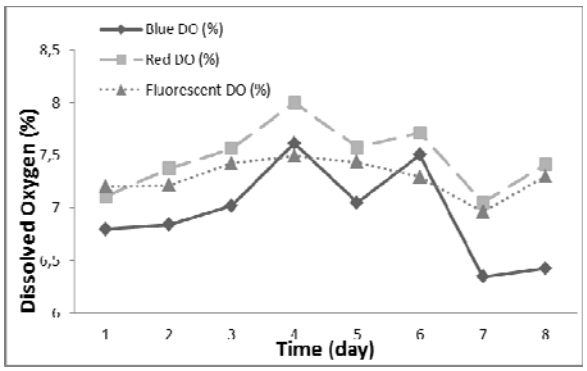

Figure 6. Evaluation of dissolved oxygen rate change with time.

Doubling Time

Doubling time is important for estimating the amount of biomass being produced by growing microalgae. For this purpose, a formula by Richmond (2004) was used. The calculated data about doubling time are shown in Table 2. In the experiment, doubling time was calculated as 907 h for blue LED light, 103 h for red LED light and 86 h for fluorescent lamps.

Table 2. Calculated doubling times for illumination sources.

Illumination source	Doubling time (h)
Blue LED	907
Red LED	103
Fluorescent	86

The size of microalgae cells was determined with ImageJ software (Wayne Rasband National Institutes of Health, USA). The most efficient illumination source in terms of cell size is the blue LED light. The microalgae cells which were grown under the blue LED light grew to as large as 16 µm in diameter. The average diameter of the microalgae under blue LED lights was 7.5 µm. In addition, most of the microalgae grown with the blue LED light were bigger than those grown with the other illumination sources. They were also the most circular in shape. The microalgae grown under the red LED lights grew to a maximum diameter of 12 µm. The average diameter of the microalgae was 6 µm in the red LED PBRs. Compared to the microalgae cells grown under blue LED lights, the microalgae cells grown under red LED lights were smaller and not as circular. The fluorescent light lit PBRs had a maximum microalgae

cell diameter as large as the blue lit PBRs, 16µm. The average diameter of these microalgae cells was measured at 7 µm smaller than those grown under blue LED light and only slightly larger than those grown under red LED light. The size and shape of the microalgae grown under fluorescent light were more variable than the ones in the two LED lit PBRs. However, the large cell size does not necessarily mean more algal biomass. The red LED lit PBR produced more biomass (2.9 g/l) even though the average cell size was smaller than that of the blue LED lit PBR shown in Figure 4. In addition, the fluorescent lit PBR produced more cells ($3.5x10^6$ cell/ml) as shown in Figure 3 than the blue LED and red lit PBRs.

Fatty acid methyl ester analysis

For the fatty acid analyzes all algae concentration grown under different illumination sources were mixed and dried together. Then 8 gram dried Chlorella kessleri was obtained. The fatty acid methyl ester profile of Chlorella kessleri is shown in Figure 7. Palmitic (C16:0), estaric (C18:0) and oleic (C18:1) fatty acids were observed with the GC. These fatty acids are suitable for biodiesel production (Sydney et al 2010).

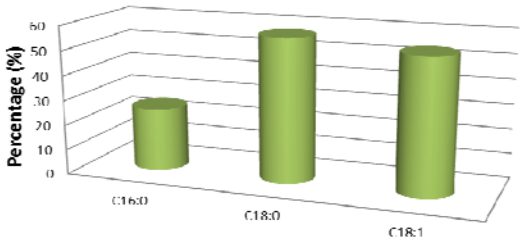

Figure 7. Fatty acid methyl ester profile of lipid extracted from Chlorella kessleri

CONCLUSIONS

Many scientists are investigating the economical production of algal biodiesel. Production of algal biodiesel requires large scale algal cultivation to reduce production costs. In this study all environmental conditions, except illumination sources were kept constant when growing Chlorella kessleri (UTEX 398) microalgae. The results showed that fluorescent light was the most efficient illumination source producing the highest number of cells. Red LED light was the most effective illumination source producing the highest biomass weight and the most efficient illumination source in terms of cell size was the blue LED light. Fatty acid composition of Chlorella kessleri indicates that Chlorella kessleri is a potential source for biodiesel production.

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