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High Density Bioreactor Cultivation of *Chlorella* sp. for Lipid Production and Bioelectricity generation

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Abstract: *The exploitation of renewable energy sources for delivering carbon neutral solutions has become challenging currently as conventional fuel sources are limited. Microalgae, in this context have recently attracted considerable interest worldwide due to their extensive application potential as they are renewable, sustainable and economic sources of biofuels, bioactive medical products and food ingredients. Algae have a high potential for converting carbon dioxide from the atmosphere into biomass, and are also being used in the development of biophotovoltaic platforms which are used to harvest solar energy for bioelectricity generation. The most challenging and crucial issues in microalgae cultivation are enhancing microalgae growth rate and product synthesis, dewatering algae culture for biomass production and treatment of biomass for lipid extraction. One of the most important parameter in algae culturing is the type of bioreactor used, the carbon dioxide availability and light intensity. In this study, two bioreactor configurations for Chlorella sp. cultivation were used with variations in the light intensity. Mixing and aeration was provided to ensure uniform distribution of nutrients, air, carbon dioxide and light penetration into the microalgal culture. Biomass concentration in the stirred tank bioreactor was 13% higher than that in the bioreactor with rectangular geometry after seven days of cultivation in the presence of carbon dioxide. Although the viscosity was found to be higher in the rectangular tank, the chlorophyll content decreased by 45%. Further the chlorella suspensions were used in a customized biophotovoltaic cell and evaluated for voltage generation.*

Keywords: *Microalgae; Photo bioreactors; Chlorella sp.; Biophotovotaic cell; Biomass, chlorophyll .*

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I. INTRODUCTION

The use of fossil fuels has been strongly related to critical problems currently affecting society, such as global warming, global greenhouse effects and pollution. Green algae are eukaryotic microorganisms capable of using sunlight as their energy source, water as their electron source and CO_2 as their carbon source to photosynthesize and produce O_2 and biomass. Algae biomass which is composed of carbohydrates, proteins and lipids, offers a carbon neutral and renewable feedstock for producing a diverse portfolio of products including biofuels, plastics, pharmaceuticals, animal feeds and fertilizers. Hence, these unicellular photosynthetic microorganisms have received great attention for use in the large-scale production of biofuels. In particular hydrocarbon feedstock production from algal lipids holds significant advantages as algae potentially have 100 to 300 times larger oil production rate than conventional agricultural crops such as canola and soy bean (Huda et al., 2017).

Depending on strain and cultivation conditions, algae can produce hydrocarbons ranging from 20 to 50% of their dry biomass weight. Cultivation of algae does not require arable land and does not displace natural CO_2 sinks, and algae can use marginal water resources such as salt water, waste water and brackish water that are unsuitable for drinking or irrigation, thereby they do not compete with freshwater resources. Traditionally algae have been cultivated as suspended cells in open raceway ponds and tubular closed photo bioreactor systems. Light intensity is one of the major limiting factors in microalgal cultivation. Light duration and intensity directly affect photosynthesis of microalgae and has influence on the biochemical composition of microalgae and biomass yield (Krzemińska et al., 2014; Huesemann et al., 2013). In modelling the outdoor or indoor algal culture system, growth rate and biomass productivity are predicted as a function of light. Light intensities vary inside the culture and reduce in culture depth which should be taken into consideration for modelling of the bioreactor or open pond system (Ye et al., 2012). Algae species vary in terms of their requirements for maximum growth and biomass accumulation. Thus, optimal light intensity needs to be determined experimentally in each case to maximize CO_2 assimilation with a minimum rate of photorespiration and as little photo inhibition as possible. A specific duration of light/dark periods is required for algal

photosynthesis. Mixing and aeration provide uniform distribution of nutrients, air and CO_2 in microalgae culture. They also enable the penetration and uniform distribution of light inside the culture and prevent the biomass from settling and causing aggregation (Show et al., 2017; Zeng et al., 2011). If all other requirements are met but there is no mixing, biomass productivity will be lowered significantly. Thus, microalgae cultures must be continuously mixed to keep all cells in suspension with free access to light. A proper mixing system in a photo- bioreactor not only enables nutrient dissolution and light penetration into the culture but also provides for efficient gaseous exchange.

Open ponds are widely used industrially due its relatively cheaper construction, maintenance and operational cost. Other advantages of open pond systems include simplistic operation and maintenance, low energy demand, and ease to scale up (White and Ryan, 2015). However, despite the large cultivation area, cultivation of microalgae from natural water has relatively lower cell concentration and thus a highly efficient harvesting method is required. Open raceway ponds are inexpensive to build and operate but suffer from small biomass concentration (usually less than 0.5 kg dry cell mass per cubic meter of liquid, (Harmon et al., 2021). Photo bioreactors are systems used to culture phototrophs such as microalgae in an enclosed system which does not allow direct exchange of material between the culture and environment. Photo bioreactors overcome several constraints faced commonly by open pond culture design like compact size when compared to open pond, therefore providing more efficient land usage (Gupta et al., 2015). They also provide a closed and highly controlled growth condition for the culture, thus able to produce a contamination free, single strain microorganism culture. Closed tubular photo bioreactor systems can cultivate the microorganisms up to 5 kg dry cell/m^3 but they are very expensive to build, operate and maintain (Torzillo and Zittelli, 2015). The operational costs for these systems mainly comes from large power requirements for continuously mixing and pumping large volumes of relatively dilute algae slurries and additionally, in the case of closed systems, from air sparging, which is necessary to remove the oxygen build up in the closed tubes to prevent photo-oxidative damage to algae. However, the bottleneck of practical usage of photo bioreactor is its limited scalability due to various design flaws, rendering it uneconomical to be used in large-scale production (Rinanti et al., 2013).
Harvesting of microalgae is one of the main parts in microalgae processing. Several studies have suggested that it makes up 20–30% of the total production cost due to high energy demand and capital cost (Uduman et al., 2010). In general, all harvesting techniques aim to remove as much culture media from the microalgae biomass to facilitate next downstream processing such as extraction of bioactive compounds. Numerous harvesting methods have been used to collect biomass, including filtration, centrifugation, flocculation, and flotation (Ana et al., 2015). For some circumstances, a combination of two or more techniques are employed to further increase harvesting efficiency (Singh and Patidar, 2018). However, one disadvantage of using microalgae is the high economic cost due to the low-yields of lipid content in the microalgae biomass. Thus, development of different methods to enhance microalgae biomass, as well as lipid content in the microalgae cells, would lead to the development of a sustainable low-cost process to produce biofuels. Many studies have reported different methods and strategies to induce lipid production to obtain higher lipid accumulation in the biomass of microalgae cells; however, there is not a comprehensive review in the literature that highlights, compares and discusses these strategies. The heterotrophic growth of *Chlorella protpthecoides* and its ability to produce lipid content of 55% and biodiesel was reported by (Miao and Qingyu, 2006). The effect of CO_2 concentration on lipid productivity can be seen from earlier research which reported that at 50 ml/min CO_2 flowrate, the lipid productivity after 17 days of nutrient deficiency reached more than 50% which is highest value when compared to lipid content using a lower CO_2 flowrate (Widjaja et al., 2008).

One of the ways to work around the shortcomings of usage of microalgae in the industry is by increasing the growth rate to compensate for their low cell density and difficulties in harvesting. Numerous equipment and technologies have been improved across the years to ramp up the production of microalgae. Although microalgae can be cultured in a highly controlled laboratory condition, it is still harder to ensure the high productivity of microalgae in large-scale production. An ideal microalgae culturing system should possess the characteristics including adequate light source, effective transfer of materials across liquid-gas barrier, simple operation procedure, minimal contamination rate, cheap overall building and production cost, and high land efficiency. The type of bioreactor used, the carbon dioxide availability and light intensity are the most important parameters in algae culturing. In the present work, two bioreactor configurations for *Chlorella* sp. cultivation were used with variations in the light intensity. Mixing and aeration was provided to ensure uniform distribution of nutrients, air, carbon dioxide and light penetration into the microalgal culture. Further photosynthetic *Chlorella* enhanced bio photovoltaic cell was investigated for the treatment of dairy waste water to produce biomass for extracting lipids and electricity generation. The aim of this study was to investigate the efficiency of biomass production in different reactor systems, lipid production and renewable sustainable bioelectricity generation

II. EXPERIMENTAL PROCEDURE

2.1 Medium and cultivation of microalagae

Chlorella algal strain was identified for the present study. The medium used for the maintenance of inoculum and cultivation of *Chlorella* sp. was NPK medium containing (% by mass): Nitrogen-20%, Nitric nitrogen-5.2%, Ammonia-3.5%, Ureic nitrogen-11.3%, Phosphorus pentoxide-20%, Potassium oxide-20%, Magnesium oxide-0.5%, Sulphur trioxide- 1%, Iron-0.02%, Manganese-0.01%, Zinc-0.01%, Boron-0.01%, Copper-0.01% and Molybdenum-0.001%. The pH was maintained at 7.5. The Bold Basal medium used had the composition as reported by (Juhari, et al., 2020)

2.2 Reactor experiments

Algal cultivation was carried out in a laboratory scale stirred tank reactor having a working volume of 2.5 l. Air flow rate was maintained using a dust and oil free compressor. Aeration was achieved by means of a ring sparger. Aeration rate was maintained at 1 lpm. A centrally mounted shaft with impellers was used for agitation. The pH and temperature of the culture medium were monitored by using probes. The initial pH of the culture medium was 7.5 and was left uncontrolled throughout the cultivation. A photoperiod of 14 h light/10 h dark cycles was maintained for each experimental run. Samples were collected at regular intervals of time and used for biomass measurements.

2.3 Biophotovoltaic cell construction and operationPurification of Derivatives

The bio photovoltaic cell contained two chambers, an anode and a cathode, each of 2 l capacity with, 18 cm length and 14 cm width (Figure 1). The pH was adjusted to around 7 using 1 N NaOH and 1 N HCl, respectively.

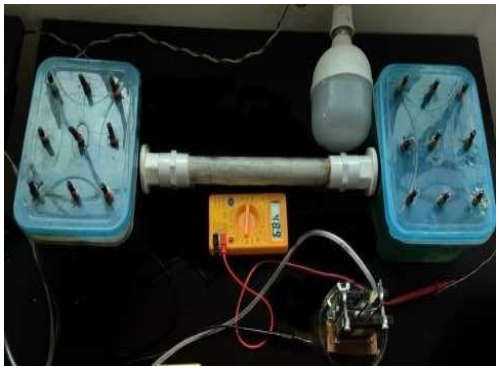


Fig. 1. Biophotovoltaic cell

Both the electrodes were made up of graphite and separated by a proton exchange membrane. A digital multimeter was connected to the system for continuous measurement of voltage. In bio photovoltaic cell under study, two sets of anolytes namely, algal strains in NPK media and algal strains in dairy industry wastewater were examined separately. Potassium ferricyanide. was used as the catholyte solution. The experiment was conducted under controlled conditions of 27 ± 1°C temperature, 3600 lm light intensity and constant aeration to support microalgal growth and photosynthesis.

2.4 Biomass estimation

Chlorella sp. were cultivated in 250 ml Erlenmeyer flasks with 100 ml working volume. Cultures were maintained at 27ºC and 100 rpm. Illumination was provided by white fluorescent light (3600 lm). For a seven-day culture continuous illumination was provided for 24 h. The culture was incubated till the stationary phase of the growth cycle was reached. Samples were collected at regular intervals and the dry weight of the biomass was estimated. Samples were collected aseptically. For *chlorella* sp., the samples were centrifuged and dried. All the experiments were carried out in triplicates.

In a photobioreactor, pH of the medium is an important factor that significantly affects the growth of the algae. Hence, to determine the effect of initial medium pH on the growth of the algae, the pH was varied in the range 6.5-8. The pH was adjusted using 0.1N NaOH or 0.1N HCl using a pH meter. Samples were aseptically collected at 24 h intervals and the dry weight of the biomass was estimated. Light intensity plays a significant role in the culture of photosynthetic organisms. Hence the effect of this parameter on the biomass yield of *chlorella* sp. was studied by growing the strain under three different light intensities of incandescent

light (1340 lm), LED light (990 lm and 3600 lm). To investigate the effect of aeration rates on the biomass yield of *chlorella* sp., air was supplied to the reactor at varying aeration rates of 1, 2, 3 and 4.0 lpm.

2.5 Cellulase Activity

Enzyme assay tubes were prepared by placing rolled filter paper strips in each of tubes. 1 ml of 0.05M citrate buffer was added with pH adjusted to 4.8, until the filter paper was saturated by the buffer. The tubes were equilibrated at a temperature of 30°C. 0.5 ml diluted enzyme was added to citrate buffer. At least two set of enzyme dilutions that released 2 mg of glucose approximately were made. 1.5 ml citrate buffer was used as the reagent blank. Enzyme control was prepared by adding 1 ml citrate buffer and 0.5 ml enzyme dilution. Substrate control was prepared by 1.5 ml citrate buffer and rolled filter paper strip of (1 x 6 cm) 50 mg Whatman No.1 filter paper (Ghose,1987). Glucose standard tubes were prepared by adding 0.5 ml of glucose dilutions and 1 ml of buffer to test tubes. The blanks, controls and glucose standard tubes were incubated at 50°C for 60 minutes. At the end of last 60 minutes DNS reagent was added to the test tubes to stop the activity. All the tubes were boiled for exactly 5 minutes in water bath. All the samples, controls, blanks and standard tubes were boiled together. After this the tubes were transferred to ice water bath. The colour was determined by measuring the absorbance against reagent blank at 540 nm (Miller, 1959).

III. RESULTS AND DISCUSSIONS

3.1. Reactor Cultivations

The culture was grown in a photo bioreactor of capacity 3Lwith continuous aeration of 1 lpm. *Chlorella* sp., was grown in NPK and Bold Basal's media for a cultivation period of 7 days. Samples were taken each day and absorbance value was noted using UV-Visible spectrophotometer at a wavelength of 685nm. The study showed that on comparing NPK and BBM media there was not much difference in the absorbance value. NPK shows a slightly better result than BBM for the seven-day culture. On the first day the absorbance value was around 0.03 and it reached a peak at 7th day with an absorbance value of 0.32. NPK media was selected because of its lower cost and easy availability as compared with BBM which required a tedious formulation and time. Figure 2 depicts the variation of absorbance in the two different media.

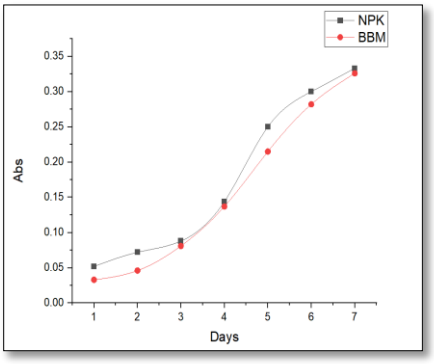


Fig. 2. Absorbance values in different media

3.2 Effect of Luminous intensity

Light is an important factor that influence the growth of algal species. The photosynthesis of algae is influenced by light intensity, which in turn impacts their growth rate. To create ATP and NADPH and other crucial chemicals for growth, microalgae need light (Pisciotta et al., 2011). The growth rate of microalgae is generally increased by increasing light intensity up to a certain point, which dependson the particular microalgae species. High light levels that reach the saturation point, however, might cause photo-inhibition. On the other hand, microalgae development will be constrained if light intensity is below the saturation point. It is well established that growing under stressful conditions, such as extremely high or low light intensities, results in a reduction in growth rate and biomass production (Laura and Paolo, 2014). The luminous intensity type depends on the area of cultivation. Different light intensities were used in the study to find the most efficient light source (Table 1). It was found that while using an incandescent bulb the heat generated due to the burning of filament was high and such high temperature was undesirable for the algal growth and the rate of evaporation was also observed high in this case. Whereas, on using LED bulb of high voltage thealgal growth showed better results with lesser evaporation rate.

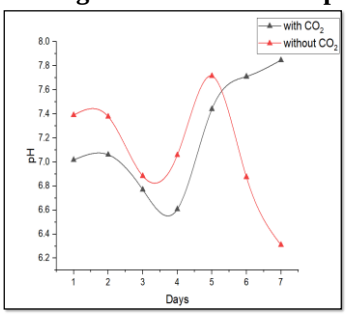
Table1 Variation of Luminous intensity

Bulb Type	Power (lm)	Algal Growth Profile
Incandescent	1340	Low
LED	990	Medium
LED	3600	High

3.3 Effect of pH

The pH of those culture provided with CO_2 showed lower value than those that is cultivated in the absence of CO_2 (figure 3). This is due to the formation of acids. To establish reproducibility, the trials were conducted three times. The downward slope observed in 3rd and 4th day may probably due to the high rate of photosynthesis that take place in 3rd and 4th day.

Fig. 3. Effect of CO_2 on pH



During photosynthesis, water got photolysed and as result a pair of electron and protons were released. These protons may be the reason for the lowering of peak. At the end the peak showed a decline in the absence of CO_2, which may be due to the depletion of nutrient media and thereby formation of an acidic environment. From this study, the optimum pH range for *chlorella* sp. was between 6.5 and 7.5 (Wang et al., 2010). The variation of pH in stirred and unstirred reactors is shown in figure 4.

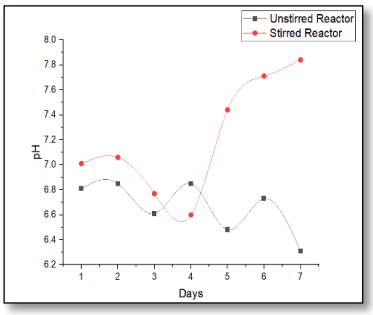


Fig. 4. Effect of mixing on pH

3.4 Effect of CO_2 on Biomass

Algal biomass is thought to be a suitable substrate for the production of biofuels. Biofuels come in a variety of forms, such as biodiesel, bioethanol, biohydrogen, and biomethane (Khan et al., 2018). Therefore, study on biomass variation is significant. Algal biomass was estimated for each day. Three samples of 1.5ml were taken in eppendorf tubes and centrifuged for 10 minutes. Biomass productivity increased in presence of CO2 than when cultivated inabsence of CO2. The biomass reached a maximum on the 3rd and 4th day of the seven-day culture after which the production of biomass declined. The peak value obtained in the 3rd and 4th day was due to the higher rate of photosynthesis during that period, however the decline in biomass was probably due to the depletion of the nutrient medium. A maximum of 0.08g/l biomass was obtained in this study (Figure 5).

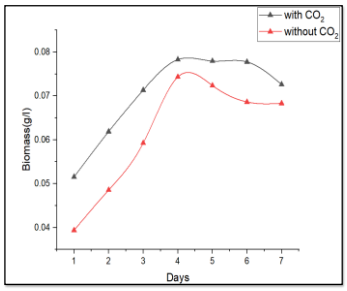


Fig. 5. Effect of CO_2 on biomass

3.4 Effect of mixing on Biomass

Study on stirred reactor and an unstirred reactor was conducted. The media and the growth conditions provided in both reactors were the same. In case of stirred reactor, 8 h continuous stirring was provided at 100 rpm. Samples from both the reactors was collected each day of a 7-day culture. Algal biomass obtained from both reactors was quantified each day after drying. The results show that stirred reactor performs better compared to unstirred reactor. In case of stirred reactor, the impellers were rotated at a defined rpm, which leads to complete mixing of the nutrients, equal distribution of the available nutrients in the media and effective utilization of nutrients by the algal cells. The biomass concentration therefore was seen to be 13% higher compared to the unstirred reactor (Figure 6). It can be concluded that stirred reactor culture to be suitable for lipid extraction as high amount of biomass can be obtained within seven days of culture.

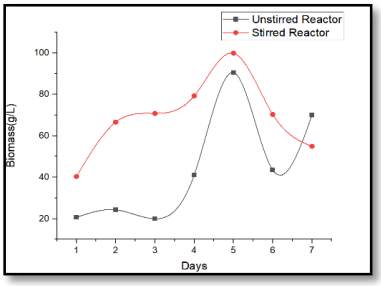


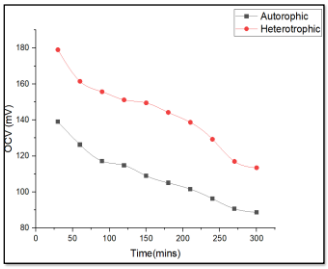
Fig. 6. Effect of mixing on biomass production

3.4 Effect of growth conditions on voltage generation

A biophotovoltaic cell with proton exchange membrane was fabricated and assembled. Graphite electrodes were used in the study. In anodic chamber algal solution was taken and in cathodic chamber potassium ferricyanide was used. Algae can be grown both in autotropic and heterotrophic modes of growth conditions. For autotrophic mode of cultivation NPK media was used in the study and for heterotrophic condition waste water from dairy industry was taken. mV.

Fig.7. Bioelectricity generation in different conditions

The heterotrophic growth condition of algae showed better bioelectricity generation over a period of 300



minutes. A peak voltage of 180 mV was observed in heterotrophic condition and later it dropped down to nearly 120 mV. In autotrophic condition the peak voltage obtained was 140 mV and decreased to 80

3.5 Effect of membrane on open circuit voltage

The protons generated during the photosynthesis of algae in biophotovoltaic cell can be transported to the cathodic side using proton selective membranes. The most commonly used membranes are Nafion 117

membrane, and proton exchange membrane. The anodic chamber of the biophotovoltaic cell was filled with algal solution and the cathodic chamber filled with potassiumferricyanide

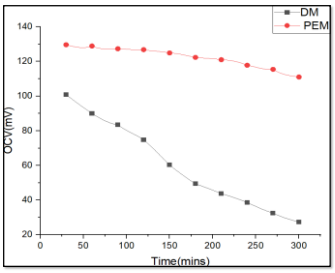


Fig. 8. Effect of membrane on bioelectricity generation

Dialysis membrane (DM) and proton exchange membrane (PEM) were used for the study, in which PEM was better and gave a peak voltage of 130 mV, where as inthe case of dialysis membrane a peak voltage of 100 mV was observed. Also this dropped drown to nearly 30 mV whereas in the case of PEM it was dropped down to 120 mV.

3.6 Effect of cathodic solutions on open circuit voltage biophotovoltaic cell using salt bridge and proton exchange membrane

In biophotovoltaic cell one of the main influencing factor for bioelectricity generation is the cathodic solution. It plays a vital role in acting as the electron acceptor. The cathodic solution acts as chemical oxidizers in order to provide an oxygen rich environment in cathode. Potassium ferricyanide and potassium permanganate were the two cathodic solutions of same molarity(1molar) was used here, in which potassium permanganate showed much higher voltage generationthan potassium ferricyanide. The

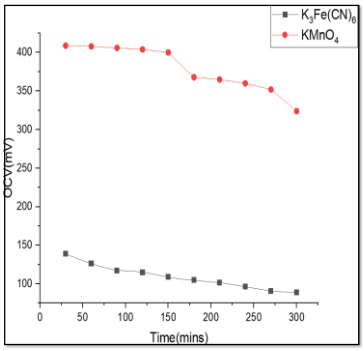


Fig. 9. Effect of cathodic solution in Biophotovoltaic cell using salt bridge

peak voltage obtained in the case of potassium permanganate was 410mV where as it was only140mV in the case of potassium ferricyanide (Figure 9).

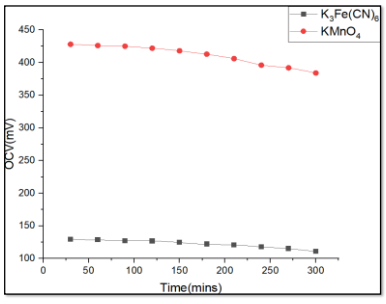


Fig.10 Effect of cathodic solution in Biophotovoltaic cell using PEM

The peak voltage obtained in the case of potassium permanganate when using proton exchange membrane was 430 mVwhereas it was only130 mV in the case of potassium ferricyanide.

3.7 Lipid Extraction

Cellulase was used for the lipid extraction. Table 2 shows the values of enzyme assisted lipid extraction at varying pH.

Table 2 Enzyme assisted Lipid extraction

Lipid Productivity (%)	Time (min)	Temperature (oC)	pH
35	90	60	3.8
32.5	90	60	4.8
25	90	60	5.8

From the table it is clear that the lipid productivity using cellulose was higher compared to solvent based extraction. A 16.35% of increase in lipid productivity was seen compared to solvent based extraction. Among the varied pH values, maximum lipid yield was obtained at 3.8 pH, 60°C and pretreatment time of 90 minutes for an enzyme dosage of 0.5mg/ml.

IV. CONCLUSION

The algal samples were cultured in two different nutrient media and two different reactors. In order to optimize the biomass productivity for a seven-day culture the factors considered were pH, luminous intensity and agitation. From the study, it was found that NPK media was more appropriate for *chlorella* sp. compared to BBM. The study conducted in two different reactors gave a conclusion that stirred reactor provided a better biomass productivity on an average of 90 mg/l. A better biomass productivity in stirred reactor was obtained when pH was maintained between 6.5-7.5, a luminous intensity of 3600 lm and with constant agitation (100 rpm). Hence this setup was considered ideal for lipid production and bioelectricity generation within a short period of time. Biophotovoltaic cell (salt bridge) and Biophotovoltaic cell (proton exchange membrane) were successfully constructed and yielded an open circuit voltage of 410 mV and 430 mV respectively. Chemical and enzymatic lipid extraction was carried out on the *Chlorella* sp. biomass obtained from the stirred reactor. Comparative study on extraction showed better results in the case of enzyme assisted extraction, at a pH 3.8. A maximum lipid production of 35% while keeping other variables constant was obtained. Further optimization has to be done to find the influencing factors for lipid production and bioelectricity generation. Algal based biophotovoltaic cell was developed in laboratory scale with good performance, however it still cannot be used in industrial applications due to several limited factors.

Conflict of interest

There is no conflict to disclose.

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REFERENCES

[1]. Huda, Q., Mohammad, R., Abdul-Sattar, N., 2017. Key issues in microalgae biofuels: A short review. Energy Procedia 142, 898-903.

[2]. Krzemińska, I., Barbara, P. S., Magdalena, T., Jerzy, T., 2014. Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess and Biosystems Engineering 37, 735-741.

[3]. Huesemann, M. H., Van Wagenen, J., Miller, T., Chavis, A., Hobbs, S., Crowe, B., 2013. A screening model to predict microalgae biomass growth in photobioreactors and raceway ponds. Biotechnology and Bioengineering 110 (6), 1583-1594.

[4]. Ye, C. P., Zhang, M. C., Yang, Y. F., Thirumaran, G., 2012. Photosynthetic performance in aquatic and terrestrial colonies of *Nostoc flagelliforme* (Cyanophyceae) under aquatic and aerial conditions. Journal of Arid Environments 85, 56-61.

[5]. Show, P. L., Malcolm ,S.Y. T., Dillirani, N., Tau C. L., Chien-We,i O., Jo-Shu, C., 2017. A holistic approach to managing microalgae for biofuel applications. International Journal of Molecular Sciences 18(1), 215.

[6]. Zeng, X., Michael, K. D., Xiao, D. C., Yinghua, L., 2011. Microalgae bioengineering: from CO_2 fixation to biofuel production. Renewable and Sustainable Energy Reviews 15(6), 3252-3260.

[7]. White, R.L., Ryan, R.A., 2015. Long-term cultivation of algae in open-raceway ponds:Lessons from the field. Industrial Biotechnology 11(4): 213-220.

[8]. Harmon, V. L., Ed, Wolfrum, Eric, P. K., Ryan, D., Lieve, M. L. L., Philip, T. P., John, M., 2021. Reliability metrics and their management implications for open pond algae cultivation. Algal Research 55, 102249.

[9]. Gupta, P. L., Lee, S. M., Choi, H. J., 2015. A mini review:Photobioreactors for large scale algal cultivation.World Journal of Microbiology and Biotechnology 31(9), 1409–1417.

[10]. Torzillo, G., Zittelli, G.C., 2015. Tubular photobioreactors. In: Algal biorefineries. Springer International Publishing, Switzerland, 187–212.
[11]. Rinanti, A., Kardena, E., Astuti, D. I., Dewi, K., 2013. Integrated vertical photobioreactor system for carbon dioxide removal using phototrophic microalgae, Nigerian Journal of Technology 32(2), 225–232.
[12]. Uduman, N., Ying, Q., Michael, K. D., Gareth, M. F., Andrew, H., 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. Journal of Renewable and Sustainable Energy 2(1), 012701.
[13]. Ana, I. B., Ana, L. G., Manuel, S., José, C. M. P., 2015. Harvesting techniques applied to microalgae: A review. Renewable and Sustainable Energy Reviews 41, 1489-1500.
[14]. Singh, G., Patidar, S. K., 2018. Microalgae harvesting techniques:A review. Journal of Environmental Management 217, 499-508.
[15]. Miao, X., Qingyu, W., 2006. Biodiesel production from heterotrophic microalgal oil. Bioresource Technology 97(6) , 841-846.
[16]. Widjaja, A., Chien, C.C., Ju, Y.H., 2008. Study of increasing lipid production from fresh water micro algae *Chlorella vulgaris* Journal of the Taiwan Institute of Chemical Engineers 40 (1) 13-20.
[17]. Juhari, A. N., Muhd, S. S., Wan, R. W. D., Tahereh, J., Mimi, H. A. B., 2020. UKM2 *Chlorella* sp. strain electricity performance as bio-anode under different light wavelength in a biophotovoltaic cell. Sains Malaysiana 49 (12), 3229-3241.
[18]. Miller, G. L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31(3),426-428.
[19]. Pisciotta, J. M., Yong, J. Z., Ilia, V. B., 2011. Role of the photosynthetic electron transfer chain in electrogenic activity of cyanobacteria. Applied Microbiology and Biotechnology 91,377-385.
[20]. Laura, B., Paolo, G., 2014. Algae: anatomy, biochemistry and biotechnology, second edition, CRC Press, Taylor and Francis Group.
[21]. Wang, C., Huan, L., Qinqi, W., Ping, W., 2010. Effect of pH on growth and lipid content of *Chlorella vulgaris* cultured in biogass slurry. Chinese Journal of Biotechnology 26 (8), 1074-1079.
[22]. Khan, M. I., Jin, H. S., Jong, D. K., 2018. The promising future of microalgae:Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories 17 (1), 1-21.

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