

Effect of Light Intensity on Algal Growth in Designed Hybrid Photobioreactor and Biodiesel Production

Shabnam Siddiqui, Y K Suneetha

Abstract: - In current scenario algal fuel is considered as the most viable solution for the depleting non-renewable fuel sources. This study demonstrates the controlled, continuous production of the microalgae species *Chlorella Pyrenoidosa* in a novel designed hybrid photobioreactor aimed to the later production of biodiesel. The reactor is operated at different light intensity and its effect on the biomass production and pH was assessed. The final biomass is harvested by centrifugation preceded by sedimentation from which the algal lipids are extracted using solvent extraction method and biodiesel by acid catalysed transesterification reaction is produced. The species population density is found to be exponential at 7130 LUX at a photoperiod of 8/16(L/D). The biomass growth in the developed design is established as a factor of 2.88 with respect to optical density. At the light intensity of 7130LUX, the developed Hybrid Photobioreactor yields a dry algal mass of 2.09g/L with a lipid productivity of 1.01g/L-day-1 and 70.62% of fatty acid methyl esters (FAME). The analysis of FAME is done using gas chromatography and a maximum of 73.4% biodiesel composition is observed.

Keywords – algal biofuel, hybrid Photobioreactor, light intensity.

I. INTRODUCTION

Petroleum, natural gas, coal, hydroelectric and nuclear is reflected as basic source of energy. In present scenario these fuels are recognized as unsustainable because of the diminution supplies and considered as key contributor for the accumulation of carbon dioxide in the environment leading to increase of global warming. Biomass, renewable fuel derived from organic matter is focused as an alternative energy source and can fix atmospheric CO₂ through photosynthesis. However the use of biomass as a fuel source is still in its infancy stage and not all the biomass are equally sustainable. There are limits to the amount of biomass that can be produced without impairing biodiversity or food stock. Infeasibility to scale up the process for large scale production and emission associated with the burning of biomass could be viewed as a limitation as well. To counter the above disadvantage the algal biomass is currently under study. Among biomass, algae have high photosynthetic efficiency and thus found to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels.

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Algae are single celled photosynthetic organisms which utilizes light energy, CO₂ and nutrients to grow and synthesize lipids, carbohydrates and protein. Algal lipids can be converted into biodiesel, carbohydrates into ethanol and proteins can be used as fertilizer. Algae are highly sustainable, inexpensive substrate, can stand broad ranges of temperature, pH, salinity and easy to cultivate. Algal biodiesel specially from microalgae shows high yield, upto 77 wt % lipid content, negligible lignocellulose biomass component, high fuel purity and possess denser energy [1].

Algae on large scale can be cultivated in open ponds or in closed systems such as photobioreactor. In current day progress, to utilize the algal biomass for biodiesel production technological implementation is needed in order to maximize the output to take the central role in meeting our energy demands. Photobioreactors are closed systems more efficient as compared to open system as light intensity and algal atmosphere for its growth can be controlled.

Many of researchers have most commonly focused on the different types of reactors such as the air lift, flat panel, bubble column or a helical reactor. The limitations of each type can be integrated with the corresponding advantages of other reactors [2]. In general, the knowledge about the influence of different sources of light and the optimal intensity required to illuminate all the cells in the reactor is a major challenge as the cells in the interiors of the reactor tend to be light shaded.

The objective of current work is to fabricate an integrated system of feasible photobioreactors and to study the relationship between the cell growth of *Chlorella Pyrenoidosa*, as influenced by the light intensity sourced from LED lights, and biodiesel production from algal biomass grown in photobioreactor.

II. MATERIALS AND METHODS

2.1 *Chlorella Pyrenoidosa*

Based on lipid content *Chlorella Pyrenoidosa* was selected for the present study. Lipid content of *Chlorella Pyrenoidosa* is 20%-51% of dry biomass [3].

2.2. Design of Hybrid Photobioreactor

Hybrid photobioreactors, a class of highly effective photobioreactors which utilizes the salient features of two or more closed systems for the economical production of different species of microalgae and compares the biomass productivity. Column reactor with helical coil was designed in this project to overcome the inefficient mixing mechanism of column reactor as well as to increase the total illumination area for faster growth of algae.

The column reactor unit is fabricated using acrylic glass material with the column dimensions of length 0.2m, breadth 0.2m and height 0.5m. This column is provided with a Sparger at the centre of its base to ensure the supply of CO₂ into the system. The illumination to this unit is done by the panel of LED's which were projected so as to give maximum light intensity of 9700LUX at the source.

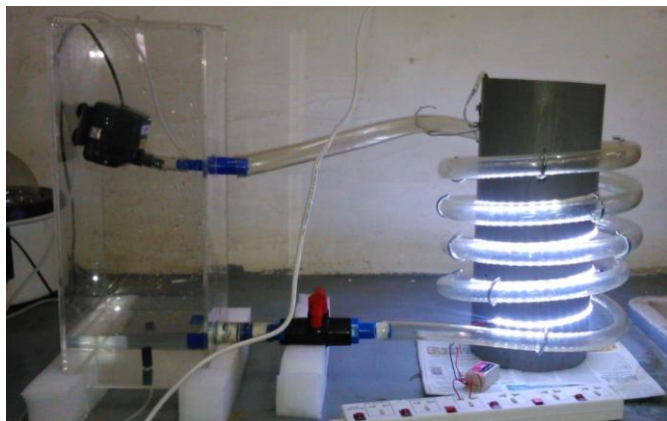


Figure 2.1 Fabricated hybrid Photobioreactor

On the other hand, the helical reactor unit is constructed using five helical parts by a 5m transparent PVC tube of 0.025 m internal diameter (id). The helical turns are placed at an elevation of 30° angle with the help of adjustable U-hooks supported on the installation pipe. The significant novelty of this design is the illumination of the helical coils at the internal coil surface as seen in figure 2.1.

The total illuminated surface area is 0.6328m² (0.24m² of the column unit and 0.3928m² of the helical unit). The volume of each helical part was 0.590L. The total volume of the system was 22.9505L. The active volume of the system was 13.95L. The volume of the column unit was nearly 6.5 times greater than that of the helical unit. The samples were collected at the sampling port using a sterilized syringe tube. The final output of the reactor was collected after every batch operation to find the total biomass yield.

2.3 Sub Culturing of Algae

Chlorella Pyrenoidosa was subcultured in 500mL EM flasks with modified Fog's media in a 1:5 ratio of algal suspension to media. Temperature maintained is usually around 25°C-30°C and pH around 6.5-7.5 [4]. Minimum light of the intensity 2000-3000 LUX should be provided (fluorescent or LED lights can be used) [5]. In the first stage of this experimentation, the algae was exposed to natural light for growth while in the next stages LED lights were installed. For the flask studies, oxygen requirements were fulfilled by natural surface aeration [6]. Within the first week media started turning slightly green. By the second week, enough algae had grown in it to give its distinct green coloration and thus considered as ready for seed inoculation.

2.4 Seed Media Preparation & Inoculation

The preparation of the inoculum culture for the microalgae cultivation in the reactor was done using the complementary modified method for algal growth as described by Allan inoculum [7].

The Fog's liquid culture media was prepared for about 12L. The sterilized 12L culture media was aseptically transferred into the hybrid photobioreactor to initiate the cultivation process of microalgae. The 2L seed inoculum prepared is then

inoculated into the photobioreactor containing the culture media. The final volume of 14L culture in the reactor is then allowed to circulate between the two units of the reactor. Similarly, the 2L microalgae culture of the 1st batch operation is used as the inoculums for the preceding batch studies.

2.5 Reactor Operating Conditions and Monitoring

The 20L hybrid photobioreactor with a working volume of 14L was sterilization for the controlled cultivation of algal. Airflow rate of 3L min⁻¹ (approx. 0.4 ppm) promoting efficient air diffusion throughout the bioreactor was maintained. The three walls of the rectangular column were illuminated by LED panels. The helical unit's illumination was maintained by the strip LED's which were draped around the support column along the same inclination angle as the helical tube. The light intensity on wall surface was adjusted according to the set objectives by manipulating the distance from the light source. A constant photoperiod of 8h light and 16h dark (8L/16D) was maintained. Samples were collected every 3 days for about 45 days, filtered and used for enzymatic assays for analysis of the product. Further the pH and biomass dry weight was analyzed. Similar batch runs of the photobioreactor were done with respect to the set objectives.

2.6 Harvesting of Algal biomass

After every batch run of the reactor i.e. after the optical density of the culture reached a value of more than 0.5 the microalgal biomass is harvested by allowing the culture volume to stand for about 2 days (48 hours) in order to sediment the biomass to the bottom of the vessel. After the defined period of time, the top clear liquid (approximately of volume 5L) was drained out using a sterilized suction pipe and the retained culture volume of about 7L was centrifuged at 10,000rpm for 10mins at 4°C. The remaining 2L of the culture was left in the system as inoculum for the next batch run.

2.7 Extraction of Lipids in Solution

The extraction of lipids from microalgae is done using the Blight and Dyer method [8]. The lipid productivity was calculated using the following equation [9].

$$P_{\text{lipid}} = (C_{\text{lipid}} \times \text{DCW}) / T$$

Where,

P_{lipid} = lipid productivity in g L⁻¹ day⁻¹,

C_{lipid} = lipid yield of the cells in g/g,

DCW = dry cell weight g/L,

T = cultivation period in days.

2.8 Biodiesel Production

In situ transesterification is performed to convert lipid into biodiesel using an acid catalyst as given by H. I. EL-Shimi et al., [10].

The biodiesel yield is calculated as below [11].

FAME yield % = (weight of hydrophobic layer/weight of microalgae sample) × 100

2.9 Analysis of biodiesel composition

The composition of biodiesel produced from transesterification of microalgae biomass is expected to consist of fatty acids range of C8 to C22 [12]. This fatty acid profile is analyzed by Gas Chromatography- mass spectrophotometry (GC-MS) which was done in the Azyme Biosciences Private Limited, Bangalore.

III. INSTALLATION AND OPERATION OF THE HYBRID PHOTOBIOREACTOR

The proposed design was successfully fabricated and installed accordingly. The design was also successfully operated with the culture to meet the research objectives. Various batches of the reactor operation was performed to test the effect of light intensity as can be seen in figure 3.1 which depicts the first batch run of the Hybrid Photobioreactor.

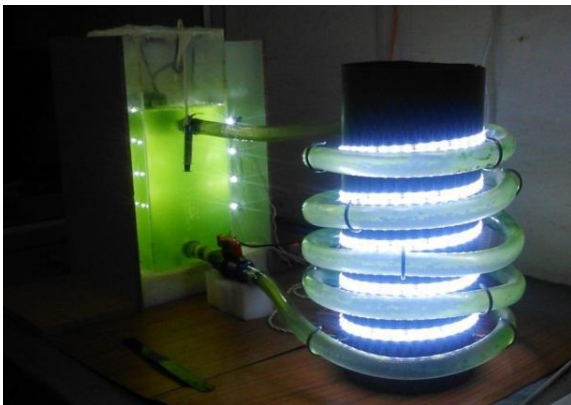


Figure 3.1 Photobioreactor operation with Batch I Culture

IV. GROWTH STUDIES IN REACTOR

4.1 Batch operation of reactor at light intensity of 3530 LUX

After the successful installation and sterilization of the photobioreactor, the culture medium and the seed inoculum were subjected to multiply their growth in the hybrid photobioreactor.

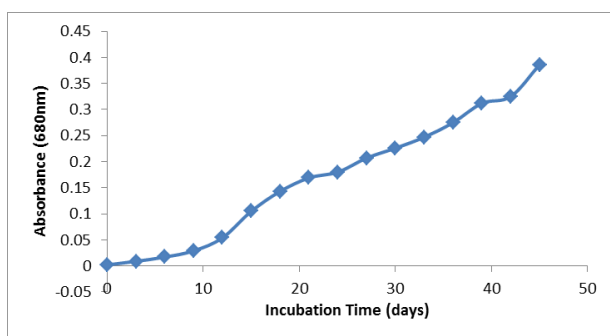


Figure 4.1 Growth curve of Batch I culture

The growth curve of the batch I culture was observed as seen in figure 4.1. The exponential phase of growth could be seen after 12 days of inoculation. However, slow growth has been seen for this batch. Also the increase in the biomass concentration with respect to optical density (680nm) was plotted and reveals that the cell concentration varies as a multiple of 2.78 of the corresponding optical density. The

biomass was harvested from the photobioreactor after 45 days of incubation and was then subjected to further analysis.

4.2 Batch operation of reactor at light intensity of 5400LUX

The operation of the batch II culture in the photobioreactor was initiated after harvesting the batch I biomass. The cell count of the residual volume of batch I was performed which was to be used as the inoculum culture for batch II. The cell count of this inoculum culture was less in comparison to the seed inoculum culture. Hence this culture was further subject to growth under optimal conditions to attain the desired cell count of 500×10^4 cells/ml which was then inoculated into the reactor for the next batch operation.

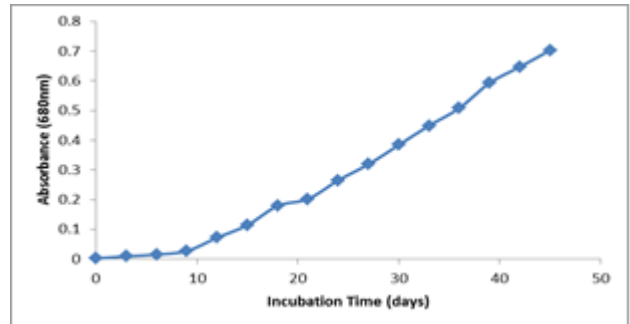


Figure 4.2 Growth curve of Batch II culture

The growth curve of was observed to show lag phase for 12 days after which the log phase of growth was observed. The culture reached an optical density of $>>0.58$ much faster than compared to batch I cultures. This shows that increase in light intensity increases the yield of the chlorella biomass.

4.3 Batch operation of reactor at light intensity of 7130 LUX

The Hybrid Photobioreactor operation for batch III culture was initiated using an inoculum culture of 467×10^4 cells/ml as counted using hemocytometer and was operated at an average light intensity of 7130LUX for a standard photoperiod of (8L/16D).

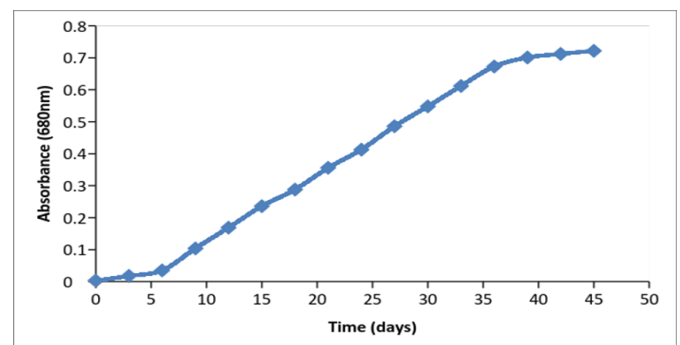


Figure 4.3 Growth curve of Batch III culture

The lag phase of this batch growth is observed to be lesser in comparison with the previous batches. This indicates that the increased light intensity is responsible to stimulate faster growth in the culture medium. Also, biomass concentration as high as 2.39g/L could be obtained at this light intensity for the developed Photobioreactor design.



The growth kinetics of the cell culture volume for all the 3 batches in the Hybrid Photobioreactor and for flask cultures was studied and reported in table 4.1

Table 4.1 Comparison between the growth rate studies of flask and different batch operations of the reactor

Growth Kinetics	Seed inoculum/ Flask studies	Batch operation of Photobioreactor		
		Batch I	Batch II	Batch III
Biomass Productivity P ($gL^{-1}D^{-1}$)	0.1005	0.0250	0.04139	0.0544
Specific growth rate K	0.034	0.0037	0.0155	0.021
Divisions per time(day) D	0.049	0.005	0.022	0.031
Generation Time (Doubling time) GT	20.286	187.617	44.843	31.847
Total yield (g /harvest)	4.02	13.56	21.32	25.08

The biomass productivity of the batch cultures as found to increase with increasing light intensities. Lowest productivity of $0.025gL^{-1}D^{-1}$ was achieved at lower intensities and highest yield of $0.054gL^{-1}D^{-1}$ was achieved at the higher light intensity. Thus the highest harvested algal mass was found to be comparable with that of flask cultures.

4.4 Effect of light intensity on biomass growth in the reactor

The plot of absorbance measured at 680nm vs. incubation time for varying light intensities. Figure 4.4.1 shows that the optical density of the culture increased significantly for higher light intensity of 7130 LUX

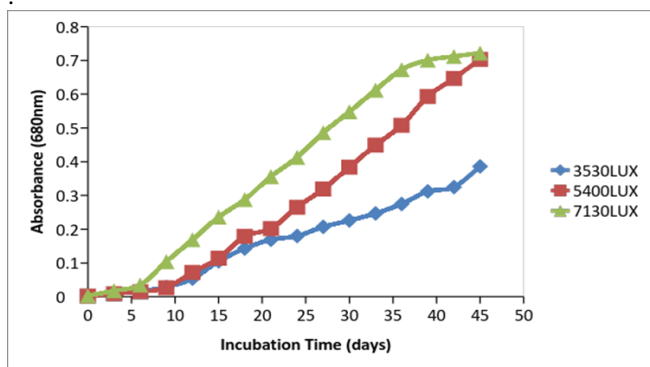


Figure 4.4.1 Absorbance measures of reactor media at different light intensities

Similar observations were noted for variation of biomass concentration with time at different light intensities. Figure 4.4.2 shows that significant boost in the biomass concentration was achieved in the log phase of culture operation at 7130LUX. This indicates that multiplicative growth increases at higher light intensities. While at lower intensities, biomass multiplication rate was very slow.

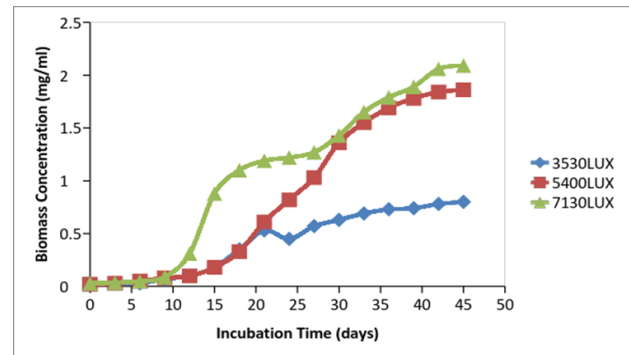


Figure 4.4.2 Biomass growth rate in photobioreactor media at varying light intensities

V. BIODIESEL PRODUCTION

5.1 Harvesting, Lipid Productivity and transesterification

After every run of reactor, the microalgae biomass was harvested and was dried to determine the dry algal biomass for each study. Afterwards the lipids were extracted from microalgae using the Blight and Dyer method and was quantified and observed that the lipid productivity increases with increasing light intensity and higher biomass content (Table 5.1). Hence, it can be stated that under optimal conditions of biomass production, improved lipid productivity can be observed. In order to produce biodiesel acid catalyzed transesterification was carried out successfully.

Table 5.1 Dry biomass, Lipid productivity and biodiesel yield of different batch operations of Hybrid

Light	Lipid	FAME yield
Intensity of	productivity	(%)
Reactor	($gL^{-1}day^{-1}$)	
operation		

3530 LUX	0.086	68.58
5400 LUX	0.098	63.25
7130 LUX	0.101	70.62

5.2 Gas chromatographic analysis of the Biodiesel produced

The gas chromatographic analysis of the samples of biodiesel obtained after every batch run of the reactor was done using CHEMITO 2000 using an FID detector with nitrogen a carrier gas with a flow rate of 1ml/min at 6000psi on a capillary column of length 30mm and 0.5mm. The report showed that the oil extracted from microalgae has fatty acid composition similar to the standard biodiesel. In the report the fatty acids constituting the biodiesel was found to be 73.4 %, 71.7% and 64.4% with decreasing light intensity respectively. As literature states that the fatty acid profiles of a species depend on growing conditions such as temperature, light and nutrients. The above study shows that higher the light intensity, higher is the biodiesel characteristic of the biomass obtained.

VI. CONCLUSION

The design of the Hybrid Photobioreactor was successfully fabricated and operated at the preferred conditions. Good results are obtained with light intensity of 7130LUX and the biomass yield with conventional reactors. It has also been observed that pH of the system does not vary significantly with light intensity. However, pH is seen to increase with increasing biomass concentration. This calls for a need of pH stabilization at higher culture multiplications.

The relationship between the optical density of the culture medium and the biomass concentration was established for each operation of the reactor. Thus the calculated value for growth in the developed design can be averaged as a function of 2.88 i.e. the biomass concentration varies as a multiple of 2.88 with respect to optical density for this design.

Lipid Productivity analysis was carried out for the three reactor operations. The result shows that lipid yield was high at higher light intensities of 7130 LUX. However, further studies can be performed to optimize the lipid productivity in the system. Further the Biodiesel yield for the biomass produced from the various operations was tested using acid catalyzed reaction and was found to have a relative yield of 70.6% biodiesel which was again obtained at higher light intensity. GC analysis of the biodiesel thus produced showed significant correlation with the standard biodiesel. Moreover the other highlighting features of the hybrid photobioreactor which is increased surface area, improved air-culture interface, improved circulation and ease of scale-up ability due to the simplicity of the design contribute to the economic production of biomass.

One of the relevant shortcomings of design was seen during the harvesting of the biomass. The adherence of the biomass to the PVC pipe was seen as a hindrance to harvesting efficiency and also could act as a light shielding area for

further batch operations. Hence a further improvisation on the design is required.

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