Journal of Academia and Industrial Research (JAIR)
Volume 4, Issue 7 December 2015

ISSN: 2278-5213

RESEARCH ARTICLE

Production of Biofuel from Micro Algae (*Chlorella pyrenoidosa*) Using Vertical Reactor System and Effect of Nitrogen on Growth and Lipid Content

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Abstract

This study deals to enhance the biomass concentration and lipid content in microalgae with one of the existing method. Microalgae, *Chlorella pyrenoidosa* was grown autotrophically in vertical bioreactor for greater efficiency. Under high light intensity, this reactor experiences less photo inhibition and under low intensity, a vertical orientation captures more reflected light. It requires less land area for installation. The *Chlorella* inoculated to vertical bioreactor showed increase in growth, also the effect of different concentrations of nitrogen source (0-0.4 g/L KNO_3) on growth and lipid content were studied. Eventually, as the nitrate concentration in the medium decreased, biomass production also decreased, however the lipid content increased. Moreover, at the same concentration of nitrate source, lipid tends to accumulate more in stationary phase in comparison to exponential phase. Highest lipid accumulation of 15% in the culture with 0.05 g/LKNO_3 was recorded. This is one-fourth of basal nitrogen source concentration. The present study emphasized that nitrogen starvation was an effective approach to enhance lipid for biofuel production.

Keywords: *Chlorella pyrenoidosa*, vertical bioreactor, nitrogen source, biofuel, biomass.

Introduction

Algae have been used as a renewable feedstock for biofuel production for many years. The efforts have not been fruitful on larger scale, thus far, since it belongs to a large group of simple photosynthetic organisms. The variety of industrial applications of algae makes it a favorite choice, such as, rapid growth, higher solar conversion efficiency than most terrestrial plants. It is harvested either batch-wise or continuously almost throughout the year. About, 50 years of research have demonstrated the ability of several micro algal species to produce several chemical intermediates and hydrocarbons which can be converted into biofuels. The three major macromolecular components chiefly obtained from micro algal biomass are lipids, carbohydrates, and proteins that can be converted into various biofuels such as alcohols, diesel, methane and hydrogen. Biodiesel is derived from organic oils, plants or animals by the process of Transesterification to obtain monoalkylesters (Demirbas, 2007). The biodiesel trans-esterification reaction is very simple:

Triglyceride + 3 Methanol ←Catalyst→ Glycerine + 3 Methyl Esters (Biodiesel)

Alkali such as potassium hydroxide acts as a catalyst in the equilibrium reaction where an organic oil or triglyceride can be processed into biodiesel (Chisti, 2007). The triglyceride is a fat, a complex molecule used by plants and animals for storing food energy. There is a high level of reductions of soot, sulphur, unburned hydrocarbon and polycyclic aromatic hydrocarbon emissions produced from diesel in comparison to biodiesel that do not give out harmful emissions (Brown *et al.,* 1993; Xu *et al.,* 2006). Minor modifications can be done in biodiesels and used as unblended or blended with fossil petroleum diesels to run engines (Ma and Hanna, 1999). Biodiesel have twice the viscosity of petroleum diesel resulting in improvement of engine life (Hankamer *et al.,* 2007). It is biodegradable and low toxic (Crookes, 2006; Schneider, 2010), like petroleum diesel biodiesel also undergo complete combustion than gasoline; hence produce a cleaner burn (Hagg, 2007). Algae show higher growth rate than food crops, thereby producing hundreds of times more oil per unit area than conventional crops such as rapeseed, palms, soybeans, or jatropha (Atabani *et al.,* 2012). Harvesting cycle of algae is 1-10 d, cultivation permits several harvests in a very short time-frame, a strategy differing from that associated with annual crops (Chisti, 2007). In addition, algae can also be grown on land unsuitable for terrestrial crops, including arid and land with excessively saline soil minimizing competition with agriculture, thus requiring lesser capital investment on land. Vertical reactor is the most efficient type of reactors for algal cultivation. Gas exchange, liquid flow and exposure of cells to light are greatly improved in a vertical air lift reactor. Using an air lift reactor helps in circulating the cultures without moving parts or mechanical pumping, hence reducing the potentials of contamination and cell damage occurring due to shear. The high and low intensities of light play major role, while high intensity of light causes vertical less photo inhibition in reactor and vertical orientation capture more reflected light under low light intensity.

Fig. 1. Vertical reactor setup.

The work is focused on choosing specific algae from the culture obtained from waste water algal sample and produce the biodiesel using vertical reactor and to determine the lipid content of the biodiesel obtained. The nitrogen starvation study was carried out to determine the growth data.

Materials and methods

Sample collection and identification: Waste water samples were collected from JP Park, Bengaluru and algae was isolated from waste water by serial dilution method. Each dilution was plated on Fogg's media. Direct microscopic observations were made and different types of algae were observed. *Chlorella pyrenoidosa* was distinctly identified and selected**.**

Shake flask culture: Microalgae culture preparation was done using Fogg's media (1949). The culture was grown in 1000 mL flask with 500 mL media and cultures were grown in a temperature controlled incubator at 25°C by providing light/dark conditions of 12:12 in rotary shaker at 100 rpm. Culture was harvested after 24 d of incubation. Measured the total biomass concentration and then transferred to vertical reactor after growing the culture for 3 weeks.

Vertical reactor setup: Vertical reactor setup was made up of polythene bag strip of length 1.5 m, width 0.533 m and thickness 0.3 m (Fig. 1). The connections were made and the motor was supplied with electricity to pump water containing the starter algal culture. About 3 weeks old culture obtained from shake flask was inoculated into the reactor designed for investigation. The culture was exposed to natural day and night photo period and the culture environment was maintained to normal temperature and the supplement of CO_2 would enhance the growth of algae.

Biomass dry cell weight (DCW) measurement: Optical densities of the samples were taken at 600 nm to determine the biomass content. The conversion factor was established by plotting the OD at 600 nm versus DCW for a series of sample of different biomass concentrations. The samples were diluted appropriately such that the OD values lie in the range of 0.2-0.9. DCW of sample was measured gravitimetrically after drying and cells were collected, post centrifugation was conducted at 3000 rpm for 10 min and washed with water. The equation for linear regression obtained is: $y = (1.038557658\ X10^{-1})\ X\ (-7.295013686\ X10^{-4})$ and $r = (9.839458706\ X\ 10^{-1})$ where, y = DCW of algal cells, x= optical density at 600 nm.

Nitrogen starvation: Chlorella pyrenoidosa was studied for growth and lipid content in different concentration of nitrate. The actual nitrogen source concentration was 0.2% L^{-1} KNO_3. The experiment was performed in quantities of 0, 0.05, 0.1, 0.2, 0.4 and 0.6 g. The effects of different nitrogen concentration on micro algal biomass and lipid content were estimated after both 24 d (exponential phase) and 30 d of inoculation (stationary phase).

Lipid extraction: Cells were harvested by centrifugation and lipid extraction was performed following the protocol of Bligh and Dyer (1959). The cells were washed once with distilled water and centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was weighed for wet weight estimation and then dried in oven at 80°C for 2 h. For 1 g of algal biomass, 2 mL of methanol and 1 mL of chloroform was added and kept for 18 h at 25°C, 1 mL of distilled water was added and vortexed in a shaker, the layers were separated by centrifugation for 10 min at 2000 rpm. The procedure was again repeated for the pellet. The two supernatants collected were allowed to stand for 2 h. The lower organic layer with lipid was transferred to pre-weighed vial (W1). Evaporation was carried out in hot air oven at 80°C for 15 min. The weight of vial was again recorded (W2) and the lipid content was calculated by subtracting W1 from W2 and expressed as dry cell weight. The oil samples were analyzed for fatty acids and hydrocarbon content and composition by GC analysis at Bangalore test house, Bangalore.

Results and discussion

The isolation of algae from waste water was conducted by serial dilution method. The microscopic examination helped in identifying *Chlorella,* the species identification was performed to confirm it as *Chlorella pyrenoidosa* (data not shown)**.** The culture was grown in 1000 mL flask in a temperature controlled incubator at 25°C by providing light/dark conditions of 12:12 in rotary shaker at 100 rpm. Culture was harvested after 30 d of incubation. It was investigated that there was an increase in biomass concentration of about 0.258 g/L from shake flask studies. The vertical reactor was setup at a higher altitude where there was good exposure to sunlight.

Fig. 2. Algae grown in Fogg's media with different concentrations of KNO_3.

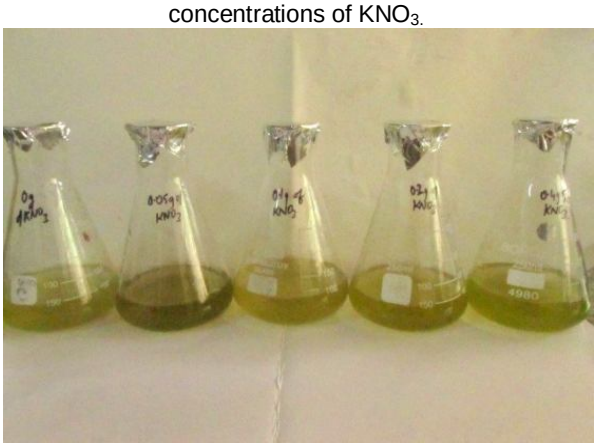


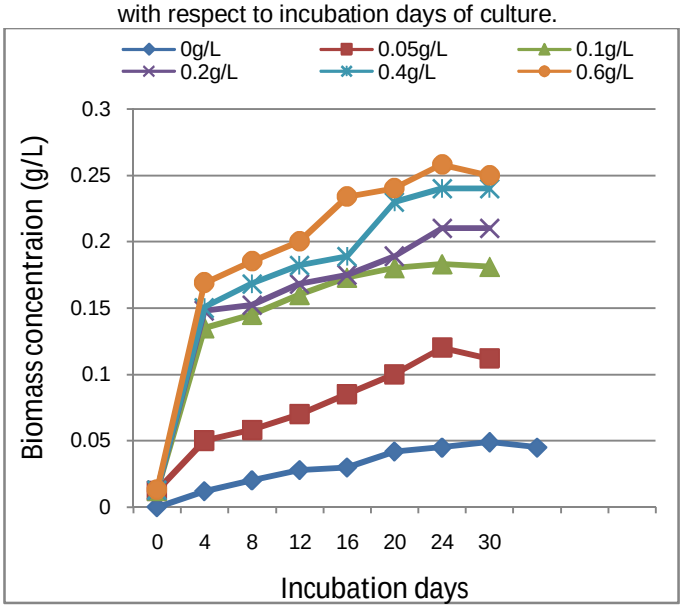
Fig. 3. Lipid extraction.

Table 1. Effects of nitrogen on biomass concentration and lipid content in stationary phase.

Conc. of KNO_3 (g/L)	Total biomass (g/L)	Lipid content (%)
0	0.049	5
0.05	0.120	15
0.1	0.183	13
0.2	0.210	12
0.4	0.240	12
0.6	0.258	10

The culture was supplied into the tank attached to a pump and electricity was supplied from source. The reactor was run for 30 d continuously until stationary phase was achieved. The culture sample after 30 d was shown in Fig. 2. *Chlorella* inoculated to vertical bioreactor showed increase in growth compared to the shake flask culture. It was found that vertical reactor is more suitable for increasing the biomass concentration of algae. The results were found to be 4.2 g/L. The nitrogen starvation study was carried out to obtain growth data of varying concentrations of nitrogen on biomass production and lipid content of the algae (Table 1).

Fig. 4. Variation of biomass cell concentration with respect to incubation days of culture.



It was found that *Chlorella pyrenoidosa* cannot grow as the nitrate source increased but increase in biomass concentration was recorded (0.258 g/L). Investigations revealed that lipid content increased as the nitrate source declined. Our study showed that lipid tends to accumulate more in stationary phase (Fig. 3, Table 1). Under the growth limiting conditions, cells had reached stationary phase, more carbon was incorporated in to carbohydrates and lipids (Fig. 4). The oil samples were analyzed for fatty acid and hydrocarbon content and composition by GC analysis for algal culture in vertical reactor and the lipid content was found to be 18%.

Conclusion

The present study suggested that enhancement of *Chlorella* biomass and lipid content increased by growing autotrophically in vertical bioreactor with suitable conditions. It also revealed that in a growth medium at initial concentration of nitrogen (0.05 g/L) there is an enhancement of lipid accumulation at stationary phase (18%).

Acknowledgements

Authors thank the Karnataka State Council for Science and Technology, SPP (Biofuel) for funding this project and also extend the thanks to Sapthagiri College of Engineering, Bangalore for providing laboratory facilities to carry out this research.

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