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Carbon Dioxide Sequestration/Utilization for Microalgal growth in Photobioreactor

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***Abstract*— Carbon dioxide is the major contributor to pollution build-up that can be circumvented if this carbon dioxide is captured and utilised for manufacturing of other products. In our present study, we aimed to cultivate the microalgae utilising renewable carbon source CO_2. We observed a rapid growth of microalgae 0.91 day^{-1} for *Nannochloropsis sp.* and 0.5 day^{-1} for *A. platensis* in stirred batch closed photobioreactor. Also, the studies on CO_2 sequestration rate by these microalgae revealed that A. platensis have 14.36% higher CO_2 sequestration rate when grown in photobioreactor.**

***Keywords*— Sequestration, CO_2, microalgae, photobioreactor.**

I. Introduction

Global warming remains a major challenge for the mankind. CO_2 is the major contributor to the global warming among all the green house gases [1]. Thermal power plants along with other industrial units cause the CO_2 emissions. There are a lot of methods to mitigate the problem including geo sequestration, ocean storage, and mineral storage. But number of problems associated with these methods such as high costs, transport of CO_2 to such locations and mainly the impact on ecology of the site used for the disposal [2]. So CO_2 capture by photosynthetic organisms and use of the biomass for valuable products becomes the focal area for researchers. Use of blue green microalgae has been proven as one of the options for efficient removal of CO_2 from the environment. It can produce up to 30 times more biomass per acre then most of the crops currently utilized for this purpose [3]. These unicellular microorganisms are photosynthetic and aquatic [4].The advantages which make them more desirable for CO_2 sequestration are that they have 10 times greater photosynthetic efficiency, higher biomass productivities, faster growth rates, higher rates of CO_2 fixation and O_2 production. Microalgae not only cleans the environment by removing CO_2 from environment but also utilized that carbon source for the production of very valuable products like food, cosmetics, pharmaceuticals and most important is biodiesel [5,6,7]. There are some important process conditions or parameters which have to be considered for the growth of microalgae and efficient CO_2 sequestration. These parameters involves energy source for phototroph which is light, essential nutrients which are carbon source, nitrogen, minerals, trace elements and vitamins, environmental factor which includes pH, temperature and salinity and processing parameters that includes proper mixing, gas transfer etc. [8]. Although cultivation of microalgae seems easy but there are many bottlenecks in this process including minimizing contamination, efficient provision of carbon dioxide, proper light intensities, controlling cultivation conditions, reducing capital and production costs, minimizing space requirements and proper mixing and aeration. These bottlenecks can be minimized by cultivating microalgae in controlled conditions of photobioreactor. For the efficient cultivation of microalgae in photobioreactor, it should possess characteristics such as high specific growth rate, ability to grow in a wide range of temperature, salinity and pH conditions, tolerance to oxygen, should not stick to the reactor surface, high photosynthetic efficiency, and ability to utilize higher light intensities. When algal farms/photobioreator placed alongside polluting units such as distilleries, thermal power plants, industrial boilers etc. they would sequester harmful flue gases like CO_2 and NOx and convert them into biomass readily processable to biofuels, proteinaceous feedstock and other high value products like enzymes, therapeutics, biopolymers etc.

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2. Materials and Methods

2.1 Microalgae cultures and their maintenance

A.Platensis and *Nannochloropsis sp*. were supplied by amazon.com. Basal salt medium was used to cultivate *A.Platensis* and *Nannochloropsis sp*. It was very important to preserve and maintain the live stocks procured of cultures. Following steps are involved in medium stock formulation and aseptic transfer of cultures. First supplied medium was dissolved in the distilled water, and sterilised. Then this was transferred to the culture bottles under laminar hood. This medium was ready for the transfer of culture. Live stock culture (when present in green colour) about 0.1mL was transferred to culture bottle. This step was performed for both cultures of *A.Platensis* and *Nannochloropsis sp*. These bottles were then incubated for 3-4 days at room temperature (nearly 30°C) and have gone through both light and dark cycles of growth. For increasing the light cycle metabolic rate, CO_2 gas was provided by placing the mouth of the bottle near the flame under laminar flow. The green colour darkened and the increase in the cell count observed after 4 days. The culture was maintained by repeated transfers to liquid and solid media. In order to preserve the culture for longer time in its

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active state, cultures was transferred aseptically to slants and agar plates for cultivating both *A.Platensis* and *Nannochloropsis*. These were also incubated at 30°C for both light and dark cycles. The transfers to liquid media were done using Erlenmeyer glass flasks of 1000ml. The flasks were filled with 200ml of culture medium and inoculated with 10% (v/v) of the strain from the stock. The flasks were kept at 25-30°C. Liquid broth cultures of both *Nannochloropsis sp*. and *A.Platensis* were used as inoculum for cultivation in photobioreactor.

2.2 Fermentation media

A.platensis was grown in Zarrouk's medium containing following components: 0.5g/l of K_2HPO_4, 2.5g/l of $NaNO_3$, 1g/l K_2SO_4, 1g/l NaCl, 0.2g/l of $MgSO_4.7H_2O$, 0.04g/l of $CaCl_2.2H_2O$, 0.01g/l of $FeSO_4$.$7H_2O$, 0.08g/l of EDTA, 4.5g/l of $NaHCO_3$ and 1ml of micronutrient solution (2.86g/l of H_3BO_3, 1.81g/l of $MnCl_2.4H_2O$, 0.222g/l of $ZnSO_4.4H_2O$, 0.0177g/l of Na_2MoO_4, 0.079g/l of $CuSO_4.5H_2O$).

Nannochloropsis sp. was grown in f/2 medium summarized below. 0.075g/l of $NaNO_3$, 0.005g/l of $NaH_2PO_4.H_2O$, 1ml of f/2 trace metal solution (3.15g/l of $FeCl_3.6H_2O$, 4.36g/l of $Na_2EDTA.2H_2O$, 0.0098g/l of $CuSO_4.5H_2O$, 0.0063g/l of Na_2MoO_4. $2H_2O$, 0.022g/l of $ZnSO_4.7H_2O$) and 0.5ml of f/2 vitamin solution (0.001g/l of Vitamin B_{12}, 0.001g/l of biotin, 200mg/l of Thiamine.HCl) were added to 950ml of filtered sea water. Vitamin B_{12} and biotin were obtained in crystalline form. When preparing the vitamin B_{12} stock solution, allow for approximately 11% water of crystallization (for each 1 mg of Vitamin $B_{12,}$ add 0.89mL dH_2O). When preparing the biotin stock solution, allow for approximately 4% water of crystallization (for each 1 mg of biotin, add 9.6mL dH_2O) [9].

2.3 Photobioreactor and its operation

2.3.1 Photobioreactor and its set up

Photobioreactor consists of a reactor vessel of height 23.5cm, diameter 14.32 cm (OD) and 13.32cm (ID), and volume 3.3ltr. Working volume is $2/3^{rd}$ of total volume i.e. 2.2ltr. Illumination was provided by 100W Halogen lamp with RED light. There is a digital monitor which displays all the values. pH was controlled by controllers which add required acid and base (KH_2PO_4 as base and K_2HPO_4 as acid) with the help of peristaltic pumps. Temperature controller maintains the temperature at 30°C. Agitation was maintained at 100rpm by agitation controller. Air and CO_2 was supplied by compressor and stack gas cylinder respectively. Reactor comprises of 2-stage impeller system in which Rushton impeller of diameter 5.5cm at the top and marine impeller of diameter 7cm at the bottom. A sintered stainless steel micro porous sparger of pore size 0.2µm is located at the bottom of the reactor vessel.

2.3.2 Photobioreactor operation

Phase-I Aerated batch

The aerated batch was carried out in photobioreactor run under controlled factors. After pouring the respective medium (f/2 for *Nannochloropsis sp.* and Zarrouk's medium for *A.platensis*) in the clean reactor, the whole reactor was autoclaved for 15min at 121°C under a pressure of 15 lb in^{-2}. After autoclaving, the console box was started and the set point of required pH (7 for *Nannochloropsis sp.* and 9 for *A.platensis*) [9, 10] and temperature was provided by putting the reactor in AUTO mode. The medium was inoculated by 10% (v/v) inoculums size. There was no agitation and carbon dioxide supply in the aerated batch because of shear sensitive cells. It was done like that so that the cells adapt reactor conditions. During those 5 days of aeration mode, *Nannochloropsis sp.* and *A.platensis* were growing at 30°C temperature, under controlled pH and in the presence of air only supplied through compressor. Only air was sufficient for the cells in this phase because micro algal cells require very less amount of CO_2 for their growth. CO_2 requirement was fulfilled by atmosphere because air comprises of 79% of nitrogen, 20.5% of oxygen and 0.5% of carbon dioxide. The pH was maintained by phosphate buffers i.e. K_2HPO_4 as acid and KH_2PO_4 as base.

Phase-II CO_2 sequestration/utilization

After 5 days of aeration mode, carbon dioxide was supplied with air in the range of 0.5-5% [11, 12]. Gas flow rate was maintained at 1.36 LPM and agitation was also fixed at 50 RPM [12, 13]. Carbon dioxide sequestration was determined by measuring the input and the output gas flow from the reactor. All the CO_2 sequestration studies were performed at the late log phase of the growth. 50ml of samples were removed after every 12 hours from the reactor. Same volume of fresh autoclaved medium was added to the reactor at the same time in order to maintain the hydrodynamic conditions in the medium. When fresh medium added to the reactor, cells rises up to get the nutrients which is advantageous in preventing the cells from settling. Light and dark cycles were maintained throughout the run. They were of equal time period i.e. 12 hours. At the end, full medium in the reactor was harvested through harvesting line. Biomass was collected by centrifuging using REMI centrifuge set at 5000 RPM for 10 minutes.

3. Analytical methods

Removed samples were analyzed to check growth of cells using Analytical Methods like optical density (OD)

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measurement, total cell count and dry cell weight measurement, lipid estimation, nitrate estimation.

3.1 Optical density measurement

Samples were used to measure the optical density (at 680 nm) using Spectrophotometer (HITACHI).

3.2 Total cell count measurement

Total cell count was measured by using Heamocytometer in which samples withdrawn were diluted to 10-1000 times.

3.3 Dry cell weight measurement

Known amount of samples were centrifuged, washed with water and taken in previously weighed and dried to constant weight aluminium foil cups, and then kept in oven (set at 70 ^{0}C) for overnight. These foil cups were dried to constant weight and weight is measured using weighing balance (SARTORIOUS). The difference in foil cup weight divided by volume of sample taken gives the dry cell weight per unit volume.

3.4 Nitrate estimation

Nitrate content was estimated by Ultraviolet spectrophotometric screening method summarized in detail in litearture [14].

3.5 CO_2 measurement

Carbon dioxide in the exit was measured by performing the titration of the exit gas collected in KOH solution (2N) provided the gas output rate with HCl (2N).

3.6 Gas composition measurement

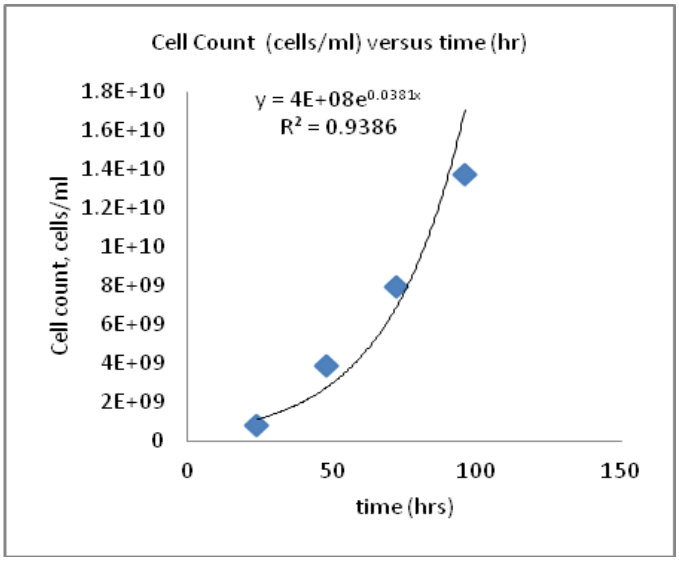
Carbon dioxide was supplied with air in the range of 0.5-5%. Gas displacement method was used for measuring the input air and CO_2 rate.

4. Results and discussion

Batch cultures of *Nannochloropsis sp.* and *A.platensis* carried out in photobioreactor at controlled pH. It has been observed that maximum growth rate of *Nannochloropsis sp.* and *A.platensis* is 0.91 day^{-1} and 0.5 day^{-1} respectively under controlled pH conditions shown in Fig-1 and Fig-2. *Nannochloropsis sp.* grows about 45% faster rate than the *A.Platensis.* We also observed the nitrate conversion ~ 80%. Studies on the two cultures showed that CO_2 sequestration rate changes with change in the CO_2 % (v/v) in the input gas ranging from 0.5 to 7%. The CO_2 % (v/v) value of about 2.9% exhibited the optimum condition where the specific sequestration rates of *Nannochloropsis sp.* and *A.Platensis* were 0.34 and 0.39 g/g/h respectively summarized in Table-1 and Table-2. Although the genotype *A.Platensis* grows slowly but have better capability for CO_2 sequestration. It means *Nannochloropsis sp.* and *A.platensis* can sequestrate 8.16 g/g/day and 9.36 g/g/day respectively of CO_2.

5. Conclusion

Nannochloropsis sp. and *A.platensis* were cultivated in a stirred batch bioreactor successfully with the build up of biomass of 2.5g/l and 3.9g/l respectively. Grown microalgae have supported the CO_2 sequestration rate of 0.34g/g/h and 0.39g/g/h for *Nannochloropsis sp.* and *A.platensis* respectively. Microalgae being a lipid producer (38-40%) have capacity to produce large number of products bio fuels, food, pharmaceuticals etc. However in our studies we focussed on cultivating the microbial cells in photobioreactor and CO_2 sequestration but such areas can explored by concentrating on high cell density cultures.



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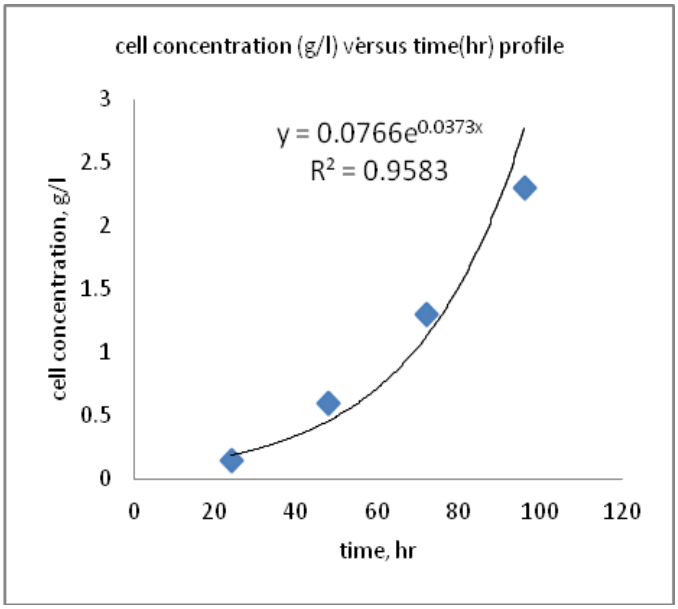

(b)

Fig-1 Growth profile of *Nannochloropsis sp.* in photobioreactor

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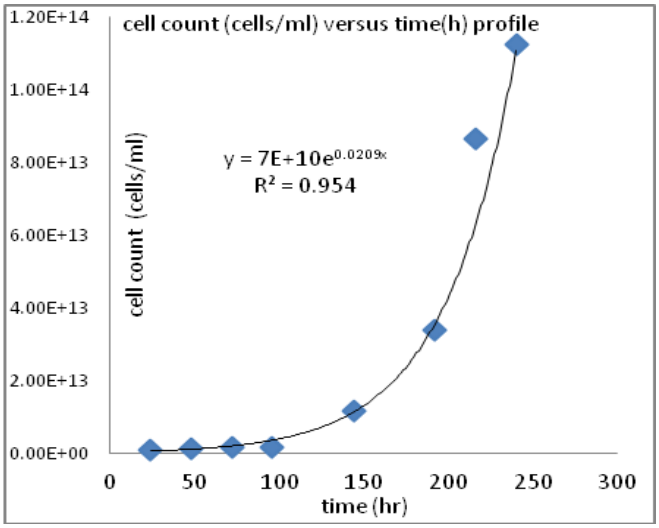

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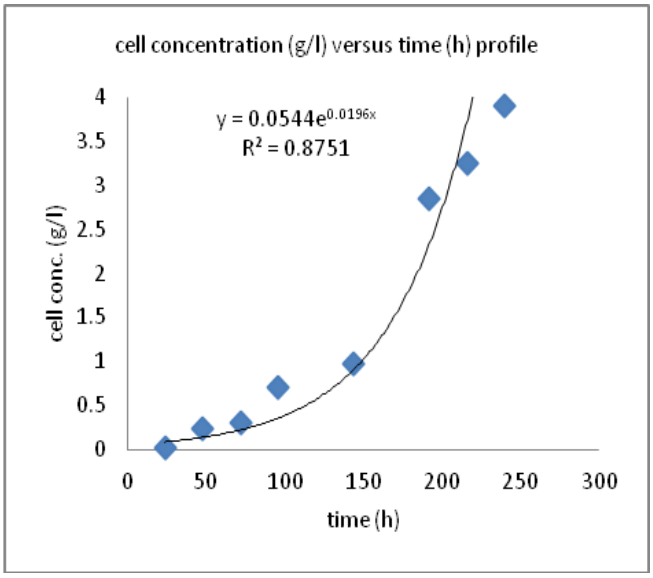

(b)

Fig-2 Growth profile of *A.platensis* in photobioreactor

Table-1 CO_2 Sequestration by *Nannochloropsis sp.*

CO_2 % (v/v)	CO_2 input Rate (g/h)	CO_2 output rate(g/h)	CO_2 sequestration rate, g/l/h	Specific CO_2 sequestration rate, g/g/h
1.357	1.904	0.8492	0.4798	0.2086
1.7094	2.398	0.8668	0.6963	0.3027
2.884	4.046	2.35	0.7713	0.3353
3.415	4.792	2.64	0.9784	0.4254
7.692	10.793	8.567	1.012	0.4401

Table-2 CO_2 Sequestration by *A.Platensis*

CO_2 % (v/v)	CO_2 input Rate (g/h)	CO_2 output rate(g/h)	CO_2 sequestration rate, g/l/h	Specific CO_2 sequestration rate, g/g/h
1.318	1.849	0.374	0.6704	0.1862
2.051	2.878	0.7304	0.9762	0.2712
2.977	4.167	1.0692	1.408	0.3911
6.593	9.251	6.006	1.475	0.4097

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