Biofixation of carbon dioxide using mixed culture of microalgae

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The objective of present study was to cultivate the mixed culture of microalgae in airlift photobioreactor in batch mode and to find out the effect of various parameters on CO$_2$ biofixation rate of microalgae. Microalgae collected from local pond were cultivated in airlift photobioreactor having working volume of 5 L for 12 d. The initial inoculum concentration and pH were 0.3 g/L and 8.4, respectively. In order to study the effect of CO$_2$ concentration and combined air-CO$_2$ flow rate on microalgae growth, CO$_2$ concentration and combined air-CO$_2$ flow rates were varied in the range of 0 to 15% and 0.2 to 2.0 L/min, respectively. The maximum biomass concentration and CO$_2$ bio-fixation rate were found to be 2.34 g/L and 373.3 mg/L, respectively, at 15% CO$_2$ concentration and combined air-CO$_2$ flow rate of 1.2 L/min.

Keywords: Airlift photo-bioreector, bio-fixation, CO$_2$, microalgae

Introduction

Global warming is a phenomenon of rising temperatures of the atmospheres and oceans. Because of global warming, the balance of global ecological system may be broken, resulting in the flooding, melting of glacier and damage to flora and fauna. According to recent studies, most of the scientists believe that the root cause of global warming is the emissions of large amount of greenhouse gas (GHG) because of global industrial activities. Carbon dioxide (CO$_2$) is the dominant GHG and its emissions have steadily risen since the industrial revolution, and are projected to increase globally by 1.3% per year$^1$. The CO$_2$ concentration in the atmosphere has increased 25% since the beginning of the industrial revolution and is still increasing$^2$. Therefore, emission mitigation strategies must be implemented to reduce the CO$_2$ emissions and thereby slowing down the effects of global climate change.

Available technologies for CO$_2$ removal/capture include physicochemical adsorbents, injection into deep oceans and geological formations, and enhanced biological fixation (or mitigation). The above mentioned methods present significant challenges, including high space requirements and potential CO$_2$ leakage over time. However, biological mitigation is the most economically feasible and environmentally sustainable technology in the long term. The slow rate is most important limitation of the biological mitigation and so the research is being carried on to enhance the biological carbon fixation. Photosynthetic microorganisms like microalgae and cyanobacteria use inorganic carbon for growth and hence can convert CO$_2$ from a point source into biomass$^3$. Microalgae have much higher photosynthetic efficiency and hence show better biomass productivity as compared to other energy crops$^4$.

It is estimated that more than 50,000 algal species exist, of which around 30,000 have been studied and analyzed$^5$. It is estimated that 1 kg of algal dry biomass requires about 1.83 kg of CO$_2$. Through photosynthetic process, CO$_2$ is absorbed by microalgae cells to support their growth by converting the carbon to carbohydrate and subsequently the carbohydrates are used to build proteins, nucleic acids and lipids$^6$. Due to their simple cell structure and fast growth rate, microalgae are expected to have CO$_2$ biofixation efficiency of 10-50 times higher compared to terrestrial plants$^6,9$.

CO$_2$ fixation by microalgae is an environmentally friendly method of removing carbon from the atmosphere, while producing many useful byproducts in the process. Microalgal growth is influenced by both physical and biotic factors$^{10}$. Light, pH, aeration$^{11}$, temperature$^{12}$ nutrient and salinity play important role in photosynthetic conversion of CO$_2$.

The strains of microalgae that have been already used by various researchers are Chlorella sorokiniana$^{12}$, Haematococcus pluvialis$^{13}$, Anabaena sp.$^{14}$, Scenedesmus obtusus$^{3,15}$, Chlorella$^{16}$, C. pyrenoidosa$^2$ and C. vulgaris$^{17}$. The optimal

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temperature range for algal growth was reported by various researchers in the range of 25-40°C\textsuperscript{18,19}. CO\textsubscript{2} biofixation rate and biomass concentration range reported by various researchers were in the range 0.174-2.22 g L\textsuperscript{-1} d\textsuperscript{-1}\textsuperscript{3,19,20} and 0.712-5 g L\textsuperscript{-1}\textsuperscript{3,18,20}, respectively.

The aim of present study was to cultivate mixed culture of microalgae in airlift photo-bioreactor in batch mode and to study the effect of varying CO\textsubscript{2} concentration and combined CO\textsubscript{2} and air flow rate. The change in pH with time at different CO\textsubscript{2} concentration and the effect of CO\textsubscript{2} concentration on CO\textsubscript{2} biofixation rate of microalgae were also studied.

**Material and Methods**

**Media Preparation and Algae Culture**

Microalgae were collected from pond located in IIT (BHU), Varanasi, India. The pH (7.83) of the water in the pond was slightly alkaline. The collected samples of microalgae were grown in 100 mL of sterile BG11 medium containing (L\textsuperscript{-1}): MgSO\textsubscript{4}\cdot7H\textsubscript{2}O 0.075 g, K\textsubscript{2}HPO\textsubscript{4} 0.04 g, CaCl\textsubscript{2}\cdot2H\textsubscript{2}O 0.036 g, NaNO\textsubscript{3} 1.5g, citric acid 0.006 g, NaCO\textsubscript{3} 0.02 g, EDTA(Na) 0.001 g, ferric ammonium citrate 0.006 g, H\textsubscript{3}BO\textsubscript{3} 2.86 g, MnCl\textsubscript{2}\cdot4H\textsubscript{2}O 1.81 g, ZnSO\textsubscript{4}\cdot7H\textsubscript{2}O 0.22 g, NaMoO\textsubscript{4}\cdot2H\textsubscript{2}O 0.39 g, CuSO\textsubscript{4}\cdot5H\textsubscript{2}O 0.079 g and Co(NO\textsubscript{3})\textsubscript{2}\cdot6H\textsubscript{2}O 0.0494 mg.

All chemicals used in this experiment were of analytical grade and purchased from Merck, India. The glassware and equipment used in the experiment were autoclaved before use. The medium was sterilized in an autoclave at 121°C for 15 min. The pH of the media after autoclaving and cooling was 8.4. The culture was cultivated at 25°C, 110 rpm in shaking incubator.

**Experimental Setup**

The schematic diagram of the fabricated experimental setup used in this study is shown in Fig. 1. For a good microalgal-CO\textsubscript{2} fixation, photobioreactor (PBR) system should have good mixing, better gas transfer and light distribution. An airlift photobioreactor having working volume of 5 L was constructed using Perspex for proper light capturing by microalgae through photosynthesis for performing CO\textsubscript{2} fixation experiments.

The setup had two concentric cylindrical columns and an air sparger (at the bottom). It was a column-like reactor that consisted of two interconnecting zones: one region where gas mixture was sparged, called riser (upflow region) and the other region was called downcomer, which did not receive the gas. CO\textsubscript{2} enriched air was introduced inside the inner column from the bottom; and degassing occurred at the top of the inner column. As the gas holdup inside, the inner column liquid was much higher than the degassed liquid outside the inner column. An upward flow of the liquid/gas mixture was obtained inside the inner column, while a downward flow of degassed liquid was generated outside of it. The biggest advantage of an airlift PBR is the excellent mixing and good exposure of cells to light.

The dispersion system of the reactor consisted of a spherical air sparger located at the bottom of the inner column. It was basically perforated tube through which CO\textsubscript{2}/air mixture was bubbled into the reactor at the base of the riser to separate the reactor volume into gassed and ungassed regions. In the PBR different concentration of CO\textsubscript{2} and air were passed after mixing. The CO\textsubscript{2} and air were obtained from...
CO₂ cylinder and air compressor, respectively. The gas was allowed to mix before passing to the PBR. The CO₂/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the air stream through two rotameters and a gas flowmeter that measured the flow rates of the carbon dioxide, the air and the mixture of gases, respectively.

Liquid circulation was induced by the hydrostatic disequilibrium caused by the density difference between the riser and the downcomer. This design generated a vertically circulating flow, which provided good overall mixing, sufficient supply of CO₂, and efficient removal of dissolved oxygen. Sunlight was provided from outside of the reactor.

The culture medium was autoclaved and poured into the PBR. The initial culture density and pH were maintained as 0.3 g/L and 8.4, respectively and experiment was carried out for 12 d. The liquid sample was collected from the sampling port at different time interval for determining microalgae cell concentration and pH. All experiments were done in triplicates.

**Analytical Techniques**

The cell concentration of culture in the PBR was determined regularly by measuring optical density at wavelength 550 nm (OD₅₅₀) using UV/VIS spectrophotometer (Elico, India) after proper dilution with deionized water to give an absorbance range of 0.01-1. The dry cell weight (DCW) of microalgal biomass was obtained by filtering 50 mL aliquots of culture through a cellulose acetate membrane filter (0.45 µm pore size, 47 mm in diam). Used filters were dried at 105°C and dry wt of blank filter was subtracted from wt of the used filter to obtain the microalgae dry cell wt. The OD₅₅₀ values were converted to biomass concentration via proper calibration between OD₅₅₀ and dry cell wt and the conversion factor was determined.

The pH was determined by a digital pH meter (model pH Tutor, Eutech Instruments, Singapore) to study the variation of pH of the culture medium with continuous CO₂ treatment.

**Determination of Growth Kinetic Parameters and CO₂ Fixation Rate**

Time-course profile of the biomass concentration (X, g L⁻¹) was used to calculate the maximum specific growth rate (µ_max, d⁻¹). The maximum biomass concentration achieved was designated as X_max (g L⁻¹).

Overall biomass productivity (P_overall, mg L⁻¹ d⁻¹) was calculated via following equation:

\[ P_{\text{overall}} = \frac{\Delta X}{\Delta t} \]  

(1)

Where \( \Delta X \) is the variation of biomass concentration (mg L⁻¹) within a cultivation time of \( \Delta t \) (d).

Specific growth rate \( \mu \) (d⁻¹) was calculated from the following equation:

\[ \mu = \frac{1}{\Delta t} \left( X_t - X_0 \right) \]  

(2)

Where \( X_1 \) and \( X_0 \) were the biomass concentration (g L⁻¹) on days \( t_1 \) and \( t_0 \), respectively.

The carbon dioxide fixation rate (mg L⁻¹ d⁻¹) was determined from the following equation:

\[ \text{CO}_2 \text{ fixation rate} = P_{\text{overall}} \times C_{\text{carbon}} \times \frac{\text{Mco}_2}{\text{Mc}} \]  

(3)

Where \( P \) is the biomass productivity (mg L⁻¹ d⁻¹); \( C_{\text{carbon}} \) is the content of carbon in the biomass (g g⁻¹), which was determined by elemental analyzer; \( \text{Mco}_2 \) is the molar mass of CO₂; and \( \text{Mc} \) is the molar mass of carbon.

The typical molecular formula of microalgal biomass, \( \text{CO}_0.46\text{H}_1.83\text{N}_0.11\text{P}_{0.01} \) was used.

**Results and Discussion**

A liner relationship \( y=0.4832x \) was found between optical density and the dry cell wt of microalgae where, \( y \) is biomass concentration (g/L) and \( x \) is OD₅₅₀.

**Effect of CO₂ Concentration on Microalgal Growth**

Industrial exhaust gases, such as, flue gases, contain 10-20% CO₂ and are rich source for microalgal cultivation and a potentially more efficient route for CO₂ biofixation. Keeping the above facts in mind, experiments were carried out at CO₂ concentrations of 0, 5, 10 and 15% at combined CO₂ and air flow rate of 1.2 L/min (Fig. 2). The results show that biomass concentration increased progressively with time in all samples and found to be 0.75, 1.42, 1.87 and 2.34 g/L at 0, 5, 10 and 15% CO₂, respectively after 12 d of cultivation. These observations clearly indicated that the yield of biomass was more in the samples having higher percentage of CO₂.
Effect of Combined Air and CO₂ Flow Rate on Microalgal Growth

One of the major challenges in biofixation of CO₂ is its poor solubility in water, which is approx 1.45 g/L at 25°C and 1 atm². The solubility of CO₂ depends on air flow rate or bubbling rate in the PBR. As higher algal growth was observed at 15% CO₂, further experiments were carried at this concentration to find out the effect of different flow rates (0.2, 0.4, 1 & 2 L/min) of combined air and CO₂ on biofixation (Fig. 3). The results show that microalgae growth rate was increased with increasing aeration rate from 0.2 to 1 L/min, but at further increase in aeration rate (2 L/min), the growth rate declined. Upto flow rate of 1 L/min, the bubbles of air and CO₂ have sufficient residence time in the liquid medium containing algae and so with increase in flow rate, higher level of turbulence and loading of CO₂ resulted in better microalgae growth. Similar results were observed in other studies¹⁹,²³ where gas liquid mass transfer coefficient was strengthened by increasing the feed gas flow rate. The flow at 2 L/min resulted in decrease of microalgae growth probably because of insufficient contact of gas bubbles with liquid media and so the most of CO₂ was released back to the atmosphere before being dissolved in the water.

Culture pH

In the present study, pH of the culture was not controlled. The average pH of the culture decreased gradually from 8.4 to 7.3 with the increase in CO₂ concentration from 5 to 15% (Fig. 4). It happened because CO₂ uptake of microalgae occurred in the form of HCO₃⁻ and CO₃²⁻ through the cell membrane, which in turn made the culture medium gradually acidic. While increasing CO₂ concentrations can lead to higher biomass productivity, the lower pH decreases the activity of carbonic extracellular anhydrase and inhibits the cell growth³.

Effect of CO₂ Concentration on CO₂ Biofixation Rate of Microalgae

The CO₂ biofixation rate (RCO₂, g L⁻¹ d⁻¹) was calculated according to Eq. (3). The results show higher rate of CO₂ biofixation by microalgae at higher concentrations of CO₂ (Fig. 5). The maximum rate of CO₂ biofixation was 373.3 mg L⁻¹ d⁻¹ at 15% CO₂ concentration.

Comparison with Traditional Monoculture of Microalgae

Application of monoculture of microalgae is more common for biofixation of CO₂. A comparison of biofixation rate of CO₂ using monoculture (from literature) and mix culture (present study) of microalgae is shown in Table 1. The data indicate that mixed culture of microalgae is equally efficient as compared to monoculture. The mixed microalgae may be a more viable and cost effective option because it is easy to grow and maintain.
References and cost effectiveness.

of CO\textsubscript{2} efficient as monoculture of microalgae in biofixation
flow rate were found favourable for biofixation of
microalgae.

Fig. 5 — Effect of CO\textsubscript{2} concentration on CO\textsubscript{2} biofixation rate of microalgae.

| Table 1 — Biofixation of CO\textsubscript{2} by various microalgae species |
|----------------|----------------|----------------|----------------|
| Microalgal species | CO\textsubscript{2} conc. (%) | Biomass productivity (g L\textsuperscript{-1} d\textsuperscript{-1}) | Ref. |
| Anabaena sp. | 10 | 1.01 | 14 |
| Chlorella pyrenoidosa | 10 | 0.26 | 3 |
| C. vulgaris | 10 | 0.25 | 17 |
| Scenedesmus obliquus | 10 | 0.29 | 3 |
| Mixed culture | 10 | 0.29 | Present study |

Conclusion

In the present study, mixed culture of microalgae was used for CO\textsubscript{2} biofixation in an airlift PBR. Higher CO\textsubscript{2} concentration, near neutral pH and moderate flow rate were found favourable for biofixation of CO\textsubscript{2}. Mixed culture of microalgae was found equally efficient as monoculture of microalgae in biofixation of CO\textsubscript{2}. Further, it may be a more viable option at large scale operation because of ease of maintenance and cost effectiveness.

References