Operational Strategies for Lab scale Horizontal Tubular Photobioreactor for Mitigation of CO$_2$ Using an Indigenous Thermophilic Microalgal Strain Geitlerinema sulphureum

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Geitlerinema sulphureum

Abstract

Anthropogenic activities leading to the industrial revolution have contributed substantially to climate change by adding carbon dioxide (CO$_2$) into the atmosphere which has caused a gradual but significant increase in the atmospheric temperature through the years. Part of the efforts to mitigate CO$_2$ involves the capture and sequestration using microalgae. Efforts were made in KET’s VG Vaze College to screen indigenously isolated thermophilic cyanobacteria for this purpose. High temperature adaptability (42°C), high CO$_2$ tolerance (23.08%), reasonably high biomass production and easy harvesting made G. sulphureum a promising candidate for the CO$_2$ sequestration in the tropical climatic conditions. The present communication deals with the optimisation of productivity of G. sulphureum in a lab scale tubular photobioreactor by altering parameters such as light regime, CO$_2$ supplementation, and nitrate and carbon source optimisation. The baseline productivity was 0.035 g/L/day before optimisation. In case of the cumulatively optimised productivity, it increased to 0.094 g/L/day with the highest reported biomass concentration of 1.29 g/L.

Keywords: Operational strategies; Tubular photobioreactor; CO$_2$ sequestration; Thermophilic cyanobacteria

Introduction

Anthropogenic activities leading to the industrial revolution have contributed substantially to climate change by adding carbon dioxide (CO$_2$) into the atmosphere which has caused a gradual but significant increase in the atmospheric temperature through the years [1]. Efforts to mitigate the greenhouse gases have revolved around lowering the production of CO$_2$ by reducing use of fossil fuel. One alternative to CO$_2$ abatement would be to capture CO$_2$ emissions and sequester them.

There are various methods of CO$_2$ sequestration including physiological, geological, oceanic, chemical and biological. Geological method for CO$_2$ sequestration does address the issue of reducing CO$_2$ levels in significant amounts but cannot be perceived as a sustainable method [2]. The cost of separation, compression and transportation by chemically mediated CO$_2$ sequestration is so costly and energy consuming, that the benefit from this process are negligible [3].

Biological CO$_2$ mitigation through higher plants and microalgae has attracted much attention as an alternative strategy, which involves fixation of atmospheric CO$_2$ by photosynthesis leading to biomass energy. Microalgal biofixation of CO$_2$ in photobioreactors has recently gained renewed interest as a promising strategy for CO$_2$ mitigation [4]. The microalgae used in CO$_2$ mitigation should be able to withstand accessory gases that accompany flue gases, scalable to industrial size photobioreactors and most importantly be capable of accumulating high value metabolites under stressing and non-stressing conditions [5].

Seambiotic israel in 2003 was the first company in the world that utilized flue gas from coal burning power stations for algae cultivation. RWE POWER Germany carried out a three year project in 2008 where a microalgal cultivation system was built in proximity to the Neideraussen power plant. Solix Biofuels focus on the cultivation systems and extraction processes for algae, along with the equipment and methods of growing microalgae in the most productive and scalable and have demonstrated the ability to produce the feedstock required to produce hundreds to thousands of gallons of lipid biocrude per year.

In India, the lead was taken by one of India’s leading cement companies in collaboration with KET’s V. G. Vaze College, Mumbai in 2008. For CO$_2$ mitigation in tropical countries, the culture used must be capable of sustaining high temperature and high light intensity. A primary level project was carried out using a species of Chlorella vulgaris (SAG 211.12) grown in a 2 litre air lift photobioreactor. The culture was found to sequester of up to 23% CO$_2$ was carried out in a 2 litre air lift photobioreactor. The main constraint in using such strains is that they do not tolerate temperatures beyond 30°C typical of Indian climatic conditions, hence deeming them unsuitable for large scale culture. Hence, efforts were made to isolate species of blue green algae from hot water springs, located in the Western Ghats of Ratnagiri, Raigad and Thane district of Maharashtra. Limnothrix redekei, Planktolyngbya crassa and Geitlerinema sulphureum were three filamentous strains that were isolated that could easily sequester about 23% of CO$_2$ without any reduction in production of biomass during culture [6]. High temperature adaptability (42°C), high CO$_2$ tolerance (23.08%), reasonably high biomass production and easy harvesting make G. sulphureum a promising candidate for the CO$_2$ sequestration in the tropical climatic conditions. Another important characteristic

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of cyanobacterium *G. sulphureum* is its halo-alkalophilic nature which enables it to grow outdoors without risk of contamination. It can be easily harvested via sedimentation and has no mucilage secretion making it easy to use on a large scale. It harbours a vibrant blue photosynthetic pigment called phycocyanin which can be used in food and cosmetic industry as an alternative to unsafe synthetic colourants. The productivity and phycocyanin content of this culture were enhanced at flask level by optimization of physicochemical parameters such as light intensity, temperature and nutrient content to achieve a productivity achieved was 0.051 g/Lday and phycocyanin yield of 71 mg/L of harvested culture [7].

The present communication deals with the optimisation of productivity of *G. sulphureum* in a 25 litre lab scale tubular photobioreactor consisting of a photosynthetic region of 8 litres and a reservoir of about 20 litres capacity by altering the light regime, CO2 supplementation, nitrate and carbonate optimisation.

**Materials and Methods**

**Microalgal species & cultivation conditions**

The freshwater cyanobacterium *Geitlerinema sulphureum* was isolated from a hot water spring located in Ganeshpuri (19.4867°N, 73.0258°E) situated in Thane district, Maharashtra, India [6]. The culture was grown in modified Zarrouk's medium [7]. The culture was initiated from solid media into 250 ml conical flask and scaled up to 1 l flasks. The culture was grown in batch mode for ten days with initial cell density of 0.45 g/L. The liquid remained constant throughout the length of the experiment.

**Bioreactor design**

The 25 litre tubular photobioreactor system consisted of two parts: the degassing unit and tubular light receiver region as seen in Figure 1. The hollow acrylic tubes are 40 mm in diameter and 72 cm long each interconnected by c - shaped bends with 90° angles. Temperature at which cultivation was carried out was room temperature and illumination of 3000 lux. The circulation of the media is carried by a bidirectional peristaltic pump ENPD 300 – Victor manufactured by Entertech India. The media is continuously aerated using an air compressor of HiSpeed Appliances India make. The flow rate of air as well as carbon dioxide was controlled by two rotameters of CVG Technocrafts India make. The percentage of carbon dioxide was varied by joining the inlet sources of both air and carbon dioxide and regulating the flow rate of both to adjust the concentration of carbon dioxide. pH, temperature, dissolved oxygen and atmospheric carbon dioxide concentrations were continuously monitored using online measurement.

**Estimation of biomass concentration**

A fixed volume of culture was harvested from the media at fixed time intervals. The biomass was separated by the media by centrifugation. Residual salts were removed by washing the biomass pellet with distilled water. The resulting biomass was filtered through preweighed Whatman paper No.1 and dried at 60°C for 24 hours. The dried biomass was weighed and results were interpreted in dry weight (g/L) [8].

**Growth studies**

Growth kinetics was studied in the tubular PBR in indoor conditions. Culture was inoculated in fresh media prepared in tap water at the beginning of each experiment with a fixed starting inoculum concentration and total volume of the media. Growth was studied in terms of dry weight and biomass productivity was calculated.

\[
P_{\text{max}} = \frac{(X_t - X_0)}{(t - t_0)} \tag{1}
\]

where \(X_t\) is the initial biomass concentration (g/L) at time \(t_0\) and \(X_t\) is the biomass concentration in (g/L) at any time \(t\) subsequent to \(t_0\).

**Experimental set –Up**

**Effect of varying inoculum density on biomass production:**

The culture was grown in batch mode with a light receiver region to reservoir region ratio of 1:2 with a light regime of 16:8 (light:dark) hours. The experimental set up involved varying the inoculum density viz. 0.25 g/L, 0.35 g/L, 0.45 g/L and 0.55 g/L respectively of dry biomass weight. The growth was monitored every 24 hours and the maximum biomass concentration and maximum biomass volumetric productivity were recorded.

**Effect of varying Light regime:**

*G. sulphureum* was grown in batch mode for ten days with initial cell density of 0.45 g/L. The experimental set up involved varying the light exposure periods sequentially (16L 8D, 20L 4D and 24L D light period) and thereby studying its effect on the productivity. The growth was monitored every 24 hours and the maximum biomass concentration and maximum biomass volumetric productivity were recorded.

**Effect of air flow rate and media circulation rate on absorption of CO2:**

*G. sulphureum* was grown in batch mode for ten days with initial cell density of 0.45 g/L. For the present culture system, the effect of flow rate of circulating media and gaseous flow rate on CO2 dissolution was undertaken and associated pH change was noted. The flow rate of the circulating medium was adjusted using a peristaltic pump wherein the volume of medium circulated was directly proportional to the number of rotation per minute. The air flow rate and CO2 flow rate were adjusted using rotameters so that the inlet concentration of CO2 (10%) remained constant throughout the length of the experiment.

**Effect of carbon dioxide supply:**

*G. sulphureum* was grown in batch mode for ten days with initial cell density of 0.45 g/L. The liquid
In the present system, when 0.25 g/L of inoculum was added to the media and the experiment was run, the culture stuck to the walls of the tubes in the light receiver region after overnight light exposure. When the media was inoculated with 0.35 g/L inoculum, though there was no lag phase, there was a negligible increase in biomass up to the first three days after inoculation (Figure 2). The increase in biomass concentration spanned throughout the entire growth period. At the end of 10 days, the maximum biomass recorded was 0.615 g/L as seen in Table 1. At this concentration, a maximum biomass volumetric productivity of 0.029 g/L/day was observed. When the inoculum concentration was switched to 0.45 g/L, the maximum biomass concentration achieved was much higher (0.802 g/L) with a maximum biomass volumetric productivity of 0.038 g/L/day. In comparison to the lower seeding density, the culture achieves a relatively higher maximum biomass concentration in a shorter span of time thereby enhancing the productivity. At a higher seeding density of 0.55 g/L, the maximum biomass concentration was achieved on day 5 was 0.789 g/L, beyond which a decline in biomass concentration is observed with a final biomass concentration of 0.7 g/L. The maximum biomass productivity achieved was 0.016 g/L/day. From the above data it could be inferred that an intermediary seeding inoculum density (0.45 g/L) is ideal to maximise the biomass productivity.

Catawatcharukul [10] studied the effect of varying inoculum density on the growth of a culture of *Spirulina platensis* in a tubular photobioreactor with light receiver tubes of 3 cm diameter and a media holding capacity of 12 litres. Light incident on the light receiver region of the photobioreactor was 60 µE m² sec⁻¹. Culture grown at concentration of 0.4 g/L achieved higher productivity of 0.086 g/L/day compared with the productivity of 0.053 g/L/day at cell density of 0.2 g/L. At 0.6 g/L, light seemed to be limited. At this cell density, the system did not reach a steady state. This is consistent with our findings that lower inoculum densities exhibit a prolonged lag phase while higher inoculum densities hamper growth in the initial period itself [10].

### Optimisation of light regime

The light regime under which microalgae is cultivated is an important factor that governs productivity of the microalgal culture. When the culture was grown with a light exposure time of 16 hours (16 L/ 8 D) at ambient concentration of CO₂, log phase was achieved till the 8th day of growth beyond which it gradually decreased. The maximum biomass productivity was 0.0381 g/Lday with a final biomass concentration of 0.802 g/L. When the light exposure was increased to 20 hours (20 L/ 4 D), log phase was achieved till the sixth day with a final volumetric productivity of 0.047 g/L/day with 0.862 g/L of biomass produced as seen in Table 2. However when the culture was exposed to continuous light (24L 0 D), it was found to be limiting to the productivity and the log phase was achieved till the 7th day. The maximal volumetric productivity declined to 0.031 g/L/day and a biomass of 0.718 g/L. The effect of varying light regimes on the growth of *G. sulphureum* was recorded in Figure 3. It was observed that increasing the light regime by four hours, did affect the productivity positively but when the light regime was increased beyond that, it negatively affected the growth as

<table>
<thead>
<tr>
<th>Light Regime (light exposure: darkness in hours)</th>
<th>Growth period (days)</th>
<th>Maximum biomass concentration Xmax (g/L)</th>
<th>Final volumetric biomass Productivity Pmax (g/L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:8</td>
<td>10</td>
<td>0.802</td>
<td>0.038</td>
</tr>
<tr>
<td>20:4</td>
<td>10</td>
<td>0.862</td>
<td>0.047</td>
</tr>
<tr>
<td>24:0</td>
<td>10</td>
<td>0.718</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Table 2: Effect of varying light regime on maximum biomass concentration and productivity.

### Results and Discussion

#### Effect of varying inoculum density on biomass production

The biomass productivity of microalgae is highly related to the growth rate, which is very sensitive to the culture conditions, such as irradiance, temperature, nutrients, CO₂ supply, and inoculum size. Optimising the inoculum concentration could shorten the lag phase and allow the culture to enter logarithmic phase earlier [9]. All these factors in turn depend upon the cultivation system and conditions used. For *G. sulphureum*, when inoculum density studies were carried out at flask level, the flasks were inoculated with 0.1 g/L, 0.2 g/L and 0.3 g/L of the culture with a total volume of culture medium 100 ml in 250 ml volumetric flasks. There was a fold increase in the dry weight of the flasks inoculated with 0.1 g/L biomass while that of 0.2 g/L and 0.3 g/L of inoculum density, increase in biomass was 2.43 and 1.83 fold respectively. At flask level, an inoculum size of 0.1 g/L was ideal for optimum productivity of *G. sulphureum* [7].

![Figure 2: Effect of varying inoculum concentration on growth of G. sulphureum.](image)

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<table>
<thead>
<tr>
<th>Inoculum Density(g/L)</th>
<th>Growth period</th>
<th>Final biomass concentration Xmax (g/L)</th>
<th>Overall volumetric biomass Productivity Pmax (g/L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>10</td>
<td>0.615</td>
<td>0.029</td>
</tr>
<tr>
<td>0.35</td>
<td>10</td>
<td>0.802</td>
<td>0.381</td>
</tr>
<tr>
<td>0.55</td>
<td>10</td>
<td>0.789</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Table 1: Effect of inoculum concentration on growth and productivity of *G. sulphureum*.

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Circulation rate was adjusted to 100 rpm. Air flow rate was maintained at 0.1 L/min and the amount of CO₂ sparged was varied to 0.01 L/min (9.09% CO₂ concentration), 0.02 L/min to (16.67% CO₂ concentration) and 0.03 L/min (23.08% CO₂ concentration). The effect of CO₂ sparged on growth of the culture was interpreted in terms of biomass productivity. The pH changes associated were also noted.

#### Effect of replacement of carbon source: *G. sulphureum* was grown in batch mode for ten days with initial cell density of 0.45 g/L. In the experiment, the carbon source i.e. sodium bicarbonate (10 g/L) usually found in modified Zarrouk’s media was replaced to sodium carbonate (3 g/L) and the effect on productivity of the culture was noted.

#### Effect of increasing nitrate concentration: *G. sulphureum* was grown in batch mode for ten days with initial cell density of 0.45 g/L. The experimental set up involved increasing the nitrate source i.e. from 2.5 g/L to 3.5 g/L. The growth studies of the culture were conducted in the presence of both standard and nitrate supplemented conditions and the productivity was compared.

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**Figures**

- Figure 2: Effect of varying inoculum concentration on growth of *G. sulphureum*.

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**Tables**

- Table 1: Effect of inoculum concentration on growth and productivity of *G. sulphureum*.
- Table 2: Effect of varying light regime on maximum biomass concentration and productivity.
Effect of varying light regime on biomass concentration of _G. sulphureum_.

<table>
<thead>
<tr>
<th>RPM (RPM)</th>
<th>Air flow rate (L/min)</th>
<th>CO₂ flow rate (L/min)</th>
<th>AVERAGE INCREASE CO₂ concentration (in ppm)</th>
<th>AVERAGE RISE IN pH</th>
<th>Temperature (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.1</td>
<td>0.01</td>
<td>STAGNATION OF CULTURE DUE TO INSUFFICIENT AIR FLOW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>0.2</td>
<td>0.02</td>
<td>453</td>
<td>558</td>
<td>8.49</td>
</tr>
<tr>
<td>80</td>
<td>0.4</td>
<td>0.04</td>
<td>439</td>
<td>594</td>
<td>8.75</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
<td>0.01</td>
<td>438</td>
<td>547</td>
<td>8.81</td>
</tr>
<tr>
<td>100</td>
<td>0.2</td>
<td>0.02</td>
<td>449</td>
<td>577</td>
<td>8.83</td>
</tr>
<tr>
<td>100</td>
<td>0.4</td>
<td>0.04</td>
<td>438</td>
<td>586</td>
<td>9.00</td>
</tr>
<tr>
<td>120</td>
<td>0.1</td>
<td>0.01</td>
<td>443</td>
<td>562</td>
<td>9.14</td>
</tr>
<tr>
<td>120</td>
<td>0.2</td>
<td>0.02</td>
<td>440</td>
<td>574</td>
<td>9.36</td>
</tr>
<tr>
<td>120</td>
<td>0.4</td>
<td>0.04</td>
<td>450</td>
<td>612</td>
<td>9.78</td>
</tr>
</tbody>
</table>

Table 3: Effect of varying air flow rate and media circulation rate on carbon dioxide absorption and pH change of media.

well as energy wastage. Hence photoperiod 20:4 was ideal.

A detailed study of the effect of the various light regimes was carried out by Lopes et al. [11]. They analysed the effect of varying photoperiod on growth and productivity of _Aphanathece microscopica Nageli_. They varied the light exposure period of the culture by two hours starting from no light exposure at all up to constant light exposure. When light exposure was increased by two hours, in each case, the biomass productivity significantly increased. The maximum productivity was observed when the culture was exposed to light continuously. Maximum values of 0.770 g/L/day, 5.100 g/L and 1.440 gCO₂/L day were obtained for volumetric productivity, maximum cell concentration and carbon dioxide fixation rate respectively, under continuous light regime.

In 2011, Sankar et al. [12] studied the effect of light regime on the green alga _Chlorella minutissima_. It was found that a maximum biomass yield of 1.4903 g/L was obtained at the end of 9 days of cultivation for cultures grown under 14 hours of light and 10 hours of darkness. Least productivity was observed for cultures grown under 18 hours of darkness. In this case the _X_acm_ was only 0.82 g/L. Several investigations have reported that light is a major limiting substrate in culture of algal systems. Aeration rates, cell concentration inside the culture system, all affect the light availability inside the photobioreactors and in turn, it affects the productivity of the algal systems.

**Effect of circulation rate of media and air flow rate on absorption of CO₂**

In the present culture system, circulation of the medium was carried out with peristaltic pump which could be adjusted to variable RPM which increases the circulation rate of the media. The medium was aerated with a mixture of compressed air and artificial CO₂ as seen in Table 3.

Initially the liquid flow rate was adjusted to 80 rpm with an air flow rate of 0.1 L/min and CO₂ flow rate of 0.01 L/min. Under these conditions, the culture began to gradually to stick to the walls of the light receiver tubes impeding the growth of the culture. As air flow was increased, the air turbulence prevented the culture from settling. At the lowest air flow rate, the concentration of CO₂ at the outlet (degassing region) was found 558 ppm as seen in Table 3. But when the air flow rate increased to 0.4 L/min, it went up to increase to 594 ppm. This rise in the outlet CO₂ suggested that the increase in flow rate inhibited the mass transfer of the gas to the medium inhibiting the absorption of CO₂ into the medium.

When the liquid circulation rate was increased to 100 rpm and the air flow rate and CO₂ flow rate were adjusted to the lowest flow rate (0.1 L/min and 0.01 L/min), the average concentration at the outlet rose to 547 ppm, the increase being 108 ppm. When the air flow rate was increased an increment in the outgoing CO₂ was seen with the outlet concentration being 586 ppm at the maximum flowrate (0.4 L/min of air and 0.04 L/min of CO₂).

When the peristaltic pump rate was set to 120 rpm, air flow rate of 0.1 L/min with CO₂ flow rate of 0.01 L/min, the outlet CO₂ concentration from 443 ppm rose to 562 ppm with an increase of 118 ppm. At 0.02 L/min CO₂ with 0.2 L/min air the CO₂ concentration at the outlet rose to 574 ppm from 440 ppm showing an increase of 132 ppm. When the air flow rate was increased to 0.4 L/min, outlet concentration rose to 612 ppm, showing a rise of 162 ppm.

It can be seen from the above data that the circulation rate of the media does not have much impact on the CO₂ absorption in the given system since the gas remains in an air pocket at the top of every tube. However, an increase in air flow rate causes a minor but gradual rise in the carbon dioxide being evolved from the system, thereby meaning that the amount of CO₂ unabsorbed does rise gradually as the residence time of the gas in the system decreases. In order to increase residence time, the air flow rate must be kept at a minimum thus allowing better gas-liquid interface encouraging greater CO₂ absorption into the culture. In present experiments, 80 rpm was not used so as to avoid stagnation of culture. Further at 120 rpm, the increase in shear causes frequent mechanical damage to the tubing thus deeming it unsuitable for practical use.

Hence for further studies flow rate of circulating medium was adjusted to 100 rpm. The flow rate of compressed air was maintained at 0.1 L/min and the flow rate of CO₂ was gradually varied.

**Effect of CO₂ concentration on biomass production of culture**

Initially in the control experiment only compressed air was supplied to the culture devoid of artificial CO₂. Table 4 describes the effect of CO₂ concentration sparged on the maximum biomass concentration and productivity of the culture. At the end of ten days at the ambient CO₂ concentration, the biomass produced was 0.802 g/L with a productivity of 0.038 g/L/day. However when the culture media was sparged with 9.09% of CO₂ (0.01 L/min CO₂ and 0.1 L/min air), there was a rise seen in the overall biomass produced with a maximum biomass concentration of 0.96 g/L and a productivity of 0.053 g/L/day. When the carbon dioxide concentration was increased further to 16%, the maximum biomass concentration at the end of the experiment was...
was 0.1091 g/L with a biomass productivity of 0.071 g/L/day. When the culture was exposed to 23% CO2, there was a slight decrease in biomass at the end of the experiment. The maximum biomass concentration was found to be 0.855 g/L with a productivity of 0.045 g/L/day.

Manjre et al. [6] screened various microalgal isolates from hot springs for their ability to grow under various concentrations of carbon dioxide and their ability to sustain it in a 2 litre air lift bioreactor. For Scenedesmus sp., grown in the absence of CO2, the final biomass produced was 0.35 g/L. As the concentration of CO2 increased to 9.09%, the biomass produced rose to 0.41 g/L. The highest biomass was obtained at 16.67% CO2, where it increased to 0.5 g/L. At 23.08% CO2 supply, the biomass concentration decreased to 0.34 g/L. Among the Cyanophycean strains screened, L. redekei and G. sulphureum showed highest biomass production. For G. sulphureum at ambient CO2 concentration biomass produced was 0.19 g/L, which rose to 0.53 g/L at 16.67%. At 23.08%, the decline in biomass was negligible and the biomass concentration was found to be 0.52 g/L indicating that most organisms not only sustained 16% CO2 but showed an increase in the biomass production up to double or triple fold.

Undu et al. [13] studied the effect of carbon dioxide supplementation on growth of Isochrysis galbana. Artificial CO2 was supplied at 5, 10, and 15% v/v during the experiments. When the culture was grown in absence of carbon dioxide, the culture was found to have 0.8 g/L of final biomass concentration. When the culture was fed with 5% CO2, it showed a maximum biomass content of 0.846 g/L and a maximum specific growth rate of 0.173. When the carbon dioxide concentration was increased to 10%, the dry weight increased to 1.097 g/L with a maximum specific growth rate of 0.192. When the culture was supplied with highest amount of CO2 (15%), the biomass concentration was found to be 0.89 g/L with a specific growth rate of 0.173. Thus the maximum growth was found to be when the media was sparged with 10% CO2.

### Effect of varying air flow rate on CO2 absorption

The rate at which the media was circulated was 100 rpm. Compressed air was supplied at the rate of 0.1 L/min. When no artificial CO2 was sparged, the CO2 concentration at the outlet was around 450 ppm. When air sparged was 0.1 L/min and the CO2 sparged was at the rate of 0.01 L/min (9.09% CO2 concentration), the amount detected at the inlet was over 5000 ppm as seen in Table 4. The average CO2 at the outlet was 540 ppm. When air sparged was at the rate of 0.1 L/min and the CO2 sparged was at the rate of 0.02 L/min (16% CO2 concentration), the average CO2 at the outlet was 675 ppm. But when the air sparged was 0.1 L/min and CO2 sparged was at the rate of 0.03 L/min (23% CO2) the amount of CO2 detected at the outlet substantially increased to 1400 ppm. Hence air flow rate of 0.1 L/min and CO2 flow rate of 0.01 or 0.02 L/min could be ideal for the present system as seen in Table 4.

### Effect of algal growth on pH of media

When G. sulphureum was grown devoid of external carbon dioxide in modified Zarrourks’ medium, the growth of the culture brought about a gradual increase in pH throughout the growth period. Initially the pH recorded on day 0 was found to be 8.45 while it rose to 10.00 on the final day of the experiment as reported in Table 4.

The pH of the media is decided by the presence and concentration of HCO3 and CO32 ions. The buffer system created by the production and consumption of these ions as follows:

\[
H_2O + CO_2 \leftrightarrow HCO_3^- \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}
\]

During microalgal photosynthesis, utilisation of CO2 leads to an increase in pH of the media while during respiration the CO2 remains unutilised due to which a decrease in pH is usually observed. When the medium was sparged with 9.09% of CO2, the pH increased from 8.36 to 9.82, the increase through the 10 days being lesser than that found when the culture was grown devoid of carbon dioxide. Similarly when the culture was supplied with 16% CO2, the increase in pH was reduced even further from 8.42 to 9.54. The increase in pH of the medium was due to growth of algae was compensated by acidity brought about by the dissolved CO2. Finally, when 23% of CO2, the increase in pH was from 8.54 on day 0 to 8.92 on the 10th day.

Reddy et al. [14] studied the effect of varying CO2 concentration on the growth of Chlorella and Scenedesmus species grown in a flat plate photobioreactor. The medium used for their cultivation was BG1 medium. It was seen that when Chlorella sp. was grown in ambient CO2 concentration, the pH increased from 7.65 to 9.26 at the end of cultivation while Scenedesmus increased from 7.83 to 10.45. When the media was sparged with 5% CO2, the pH of the media dropped to 6.91 for Chlorella while for Scenedesmus it was found to be 7.18. When the CO2 concentration was increased to 20% CO2, the pH of the media decreased to 6.38 for Chlorella sp. while for Scenedesmus it decreased to 6.51.

### Role of alkaline pH of media in carbon dioxide absorption

CO2 in the gaseous phase is sparingly soluble in water at atmospheric pressure. As the aqueous medium turns acidic, the solubility of CO2 in the medium decreases even further. However, if the pH of the medium is alkaline, the solubility of carbon dioxide increases. Equilibrium can be brought about by providing a carbonate-bicarbonate buffer to the system such that the consumption of CO2 by the microalgae would lead to increased dissolution of the atmospheric CO2 into the media thereby increasing the consumption of CO2.

Gonzalez Lopez et al. [15] proved this theory by providing a carbonation unit to the media into which flue gases were sparged. By continuous bubbling of flue gases into a medium devoid of a carbonation buffer system, the media becomes acidic and maximum CO2 utilization efficiencies of 8.1% and 4.2% were reported. By on-demand injection, the flue gases are injected to control the pH of the culture at the optimum of the microorganism and maximum CO2 use efficiency of 32.8% was reported. In both cases, the use of CO2 absorption must be enhanced by adequate design of carbonation units, for which the knowledge of mass transfer and gas exchange phenomena into the culture was deemed necessary.

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**Table 4:** Effect of varying carbon dioxide percentage on the outlet CO2 concentration, pH change and biomass productivity of the culture medium.

<table>
<thead>
<tr>
<th>CO2/AIRFLOW RATE</th>
<th>% CO2</th>
<th>INLET CO2 (in ppm)</th>
<th>OUTLET CO2 (in ppm)</th>
<th>pH of media</th>
<th>Biomass Concentration (g/L)</th>
<th>Biomass Productivity (g/L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0%</td>
<td>450 ppm (Ambient conc.)</td>
<td>450 ppm (Ambient conc.)</td>
<td>8.45 – 10.00</td>
<td>0.802</td>
<td>0.038</td>
</tr>
<tr>
<td>0.01/0.1</td>
<td>9.09%</td>
<td>&gt;5000</td>
<td>540</td>
<td>8.36 – 9.82</td>
<td>0.96</td>
<td>0.053</td>
</tr>
<tr>
<td>0.02/0.1</td>
<td>16.67%</td>
<td>&gt;5000</td>
<td>675</td>
<td>8.47 – 9.54</td>
<td>0.1091</td>
<td>0.071</td>
</tr>
<tr>
<td>0.03/0.1</td>
<td>23.08%</td>
<td>&gt;5000</td>
<td>1400</td>
<td>8.52 – 8.92</td>
<td>0.855</td>
<td>0.045</td>
</tr>
</tbody>
</table>
When the culture was exposed to 20 hours of daily light and 10% CO₂ supply, the maximum biomass concentration of 1.024 g/L. But it was not significantly different from when the culture was grown with carbon dioxide supply of 10% and 16 hours of light exposure. It can clearly be seen that the provision of carbon dioxide supply overrides the effect of the increased light exposure. Therefore, 16L 8D with 10% CO₂ supply was used for further experiments. Thus the process of CO₂ sequestration becomes cost effective as well as saves light energy becoming a more energy efficient process. A comparative evaluation of the growth curves under varying carbon dioxide and light regimes can be seen in Figure 4.

Sen et al. [17] studied the growth of marine microalgae Chlorella vulgaris under different light and carbon dioxide regimes. Four combinations of light regimes and CO₂ supply were used. 24 hour light phase and 24 hours CO₂ supply, 12 hour light phase and 24 hour CO₂ supply, 24 hour light phase and 12 hour CO₂ supply and finally 24 hour light phase with no CO₂ supply. C. vulgaris reached a maximum of 1×10¹⁰ cells/ml on 21st day in culture under continuous illumination and CO₂ supply at 25%. In cultures with 24 hours illumination and 12 hours CO₂ supply, maximum cell density was 5×10⁹ cells/ml. The least cell density was measured when cells were grown under continuous illumination without CO₂ with only 275×10⁶ cells/ml implying that the effect of CO₂ supplementation superseded the effect of longer light exposure periods on growth of the culture.

Effect of carbonate source and concentration on productivity

Modified Zarrouk’s medium contains a very high amount (10 g/L) of sodium hydrogen carbonate (NaHCO₃). An experiment on G. sulphureum was carried out at flask level to observe whether lowering the carbonate concentration had any effect on the productivity. It was found that lowering the sodium hydrogen carbonate concentration was a deterrent to the growth of the culture. The carbon source was then replaced by sodium carbonate. Four concentrations of Na₂CO₃ (3.12 g/L, 6.24 g/L, 9.3 g/L as well as 12.5g/L) were used. At 3.12 g/L Na₂CO₃ concentration the productivity was found to be 0.035 g/Lday. When the concentration was increased to 6.24 g/L Na₂CO₃ concentration and 9.3 g/L Na₂CO₃ concentration, the increase in productivity was 0.045 g/Lday and 0.049 g/Lday respectively. The culture grown in 12.5 g/L of Na₂CO₃ showed the highest productivity i.e. 0.056 g/Lday as compared to the culture grown in modified Zarrouk’s medium containing 10g/L of NaHCO₃, i.e. 0.026 g/Lday [7].

When medium is prepared on a larger scale, high concentrations of nutrient salts mean increase in cost. Keeping this as criteria, sodium carbonate (3.12 g/L) was used as an alternate carbonate source to sodium hydrogen carbonate. It was found that the maximum biomass concentration obtained in case of 3.12 g/L of sodium carbonate was 0.828 g/L while that at standard concentration of sodium hydrogen carbonate was 0.802 g/L with the productivity being 0.038 g/Lday and 0.0428 g/Lday respectively as seen in Table 6. It could be said that the productivity in this case is almost equal to the productivity seen in standard medium which utilises almost thrice the concentration of sodium hydrogen carbonate. When sodium carbonate was used as the carbonate source and the culture was grown under all other optimised conditions (16 hours light and 10% CO₂ supply), the maximum biomass concentration achieved was 1.078 g/L as seen in Figure 5 while the biomass productivity was 0.0692 g/Lday whereas cultures in standard Zarrouk’s medium when exposed to same conditions, the biomass was 0.96 g/L and the productivity was 0.055 g/Lday.

Sounndaripandian et al. [18] studied the effect of varying carbonate concentration on productivity.
The least biomass production was observed with CaCO₃. In general, concentrations i.e. 1.5 g/L, the productivity was very negligible as seen at flask level. At 2.5 g/L of sodium nitrate, a productivity of 0.0154 g/L/day was observed. With an increase in nitrate, the biomass concentration as well as the productivity increased. At 3.5 g/L, a productivity of 0.023 g/L/day was observed. At the highest concentration of 4.5 g/L, the increase was not as significant as compared to that at 3.5 g/L, the biomass productivity was 0.025 g/L/day. Hence, 3.5 g/L of sodium nitrate may be utilized for optimized biomass production.

When the culture was grown in a reactor with 24 litre working volume, maximum biomass concentration of G. sulphureum under standard conditions with a nitrate concentration of 2.5 g/L in the medium was found to be 0.802 g/L with a productivity of 0.0381 g/L/day as seen in Table 7. When the nitrate concentration was increased to 3.5 g/L, the maximum biomass concentration rose to 1.02 g/L with a productivity of 0.0621 g/L/day. When the medium was supplemented with 3 g/L of Na₂CO₃ and 3.5 g/L of NaNO₃ with a 16 hours light period and 10% supply of CO₂, the maximum biomass produced was found to be 1.29 g/L with a productivity of 0.094 g/L/day; 2.47 times that of the original productivity while the media supplied with 3 g/L Na₂CO₃, 2.5 g/L NaNO₃, 16.8 photoperiod and 10% CO₂ supply, the biomass production seen was 1.078 g/L as seen in Figure 6 with a productivity of 0.0692 g/L/day.

**Singular and cumulative parameter optimisation**

It can be clearly seen from Figure 7 and 8 that cumulative optimisation strategy worked out positively in regards to the productivity of the culture. If each optimised parameter were compared singularly, it could be seen that the optimisation of nitrates showed the most enhancement with a productivity of 0.0676 g/L/day and a maximum biomass concentration of 1.07 g/L as seen in. This was followed by carbon dioxide supplementation wherein the productivity rose to concentration on Spirulina platensis strains. Among the different carbon sources, Na₂CO₃ significantly enhanced the biomass production while the least biomass production was observed with CaCO₃. In general, the biomass produced by all the cultures was significantly higher when NaHCO₃ of the medium was replaced with Na₂CO₃ irrespective of the inoculation periods. Among the cultures, CS-1 produced the highest biomass (22.50 mg mL⁻¹) while S-10 registered a least biomass (14.15 mg mL⁻¹) at 30 days after inoculation when Na₂CO₃ was used as a sole carbon source.

**Effect of nitrate source and concentration on productivity**

Spirulina medium contains 2.5 g/L of sodium nitrate as standard concentration of nitrate. Studies conducted on of varying the concentration of nitrates on the growth and productivity of Geitlerinema sulphureum at flask level, it was observed that at low nitrate concentrations i.e. 1.5 g/L, the productivity was very negligible as seen

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**Table 7: Effect of varying nitrate supplementation on biomass concentration and productivity of G. sulphureum.**

<table>
<thead>
<tr>
<th>Light Regime</th>
<th>CO₂ Supply &amp; Concentration</th>
<th>Nitrate Concentration</th>
<th>Maximum Biomass Concentration X_{max} (g/L)</th>
<th>Maximum Volumetric Biomass Productivity P_{max} (g/L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:8</td>
<td>10 g/L NaHCO₃, 2.5 g/L Na₂CO₃</td>
<td>0.802</td>
<td>0.0381</td>
<td></td>
</tr>
<tr>
<td>16:8</td>
<td>10 g/L NaHCO₃, 3.5 g/L Na₂CO₃</td>
<td>1.02</td>
<td>0.0621</td>
<td></td>
</tr>
<tr>
<td>16:8</td>
<td>10% Na₂CO₃, 3.5 g/L NaNO₃</td>
<td>1.078</td>
<td>0.0692</td>
<td></td>
</tr>
<tr>
<td>16:8</td>
<td>10% Na₂CO₃, 3.5 g/L NaNO₃</td>
<td>1.29</td>
<td>0.094</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 5:** Estimate of carbonate supplementation on increase in biomass concentration of G. sulphureum.

**Figure 6:** Estimate of nitrate supplementation on increase in biomass concentration of G. sulphureum.

**Figure 7:** Estimate of singular optimisation on biomass productivity of G. sulphureum.

**Figure 8:** Estimate of cumulative optimisation on biomass productivity of G. sulphureum.
0.055 g/Lday with a maximum biomass concentration of 0.96 g/L. When each parameter was cumulatively optimised, the enhancement of productivity showed a cascading effect. The baseline productivity was 0.035 g/Lday after optimisation of the light receiver volume to reservoir volume ratio and inoculum size of the culture to be used was standardised. When the light regime was optimised it could be seen that twenty hours of light exposure singularly enhanced the productivity the most i.e. 0.047 g/Lday. But when optimised light regime was coupled with carbon dioxide supplementation, it was seen that carbon dioxide supplementation overrode the effect of increasing the light exposure time since the culture gave a productivity of 0.055 g/Lday and a maximum biomass production of 0.96 g/L while in case of the culture being grown with four hours extra light exposure and carbon dioxide supplementation, the maximum biomass produced was 1.002 g/L with a productivity of 0.067 g/Lday. Further though changing the carbonate source and concentration did not singularly enhance the productivity i.e. it was found to be 0.0428 g/Lday with a maximum biomass production of 0.828 g/L, when coupled with optimised light regime and carbon dioxide supply it showed a productivity of 0.069 g/Lday with a maximum biomass concentration of 1.07 g/L. Finally when the culture was supplemented with higher nitrate concentration i.e. 3.5 g/L, the final biomass concentration rose to 1.07 g/L with a productivity of 0.067 g/Lday. In case of the cumulatively optimised productivity, it increased to 0.094 g/Lday with the highest reported biomass concentration of 1.29 g/L.

From the graph above, it can be clearly seen that the application of optimisation strategies has a positive effect on the enhancement of the exponential phase and delay of the stationary phase. Before optimisation, the exponential phase began to wane at the seventh day of growth itself. The carbon dioxide supplementation lengthened the growth phase to the 8th day and along with optimised light exposure it went up to the 9th day. When the culture was supplemented with optimised nitrate as well as carbonate, the growth phase lasted the entire span of the experiment.

Conclusion

Optimisation strategies for a 50 L tubular photobioreactor were carried out. The inoculum density of 0.45 g/L was chosen to carry forward the rest of the experiments. Varying the light regime led to the understanding that 20 hours of daily light exposure could increase the productivity of 0.38 g/Lday to 0.047 g/Lday. However supplementation of carbon dioxide to lower light exposure regime overcame the same with a productivity of 0.053 g/Lday. The supplementation for CO₂ was preferred over increasing the light exposure time so as to reduce energy consumption. From the CO₂ absorption studies, it was found that the change in media circulation rate did not significantly affect the exit CO₂ concentration of the system. Further studies on the effect of increasing concentration of CO₂ led to the understanding that 16% favoured the best productivity. But studies using approximately 10% CO₂ concentration were carried since it is the average concentration of CO₂ found in most stack gas emissions. Subsequent inorganic carbon source optimisation led to the use of sodium carbonate, which along with other optimised parameters led to a productivity of 0.0692 g/Lday. Further a slight increase in the nitrate led to the highest productivity of 0.094 g/Lday. Cumulatively optimising these parameters showed greater effect than all parameters when singularly optimised.

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References