Research Article

Operation of Photobioreactor on Refinery Gases for CO₂ Sequestration and Fatty Acid Production Using Algae

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Abstract

India over 80% of its energy needs are met through fossil fuels. India experienced dramatic growth in fossil fuel fed CO₂ emissions averaging to 5.7% per year and becoming world third largest fossil fuel emitting country. Microalgae are capable of capturing green house gas- CO₂ and converting to fatty acid. CO₂ sequestering algae identified as Chlorococcum sp was isolated from environment for fatty acid production. Flue gas from refinery with 10-15 % CO₂ has been studied for CO₂ sequestration in photobioreactor. The biomass and lipid productivity for Chlorococcum sp. with 0.1% of CO₂ from flue gas were 139 mg/l/d and 81.2 mg/l/d respectively in photobioreactor.

Keywords: CO₂ sequestration, bench scale laboratory reactor, algae, lipid production, flue gases

INTRODUCTION

The world population has been growing rapidly and has nearly doubled in the last fifty years. This rapid growth has been accompanied by economic development both of which have resulted in high energy demand. Fossil fuels, coal oil and gas have been the major sources which have supplied this energy demand for a long time. However limited availability of these sources coupled with the adverse environmental impacts associated with their extraction and use have prompted the search of other renewable energy sources to meet the future energy demands. Several renewable energy sources such as solar, wind, tidal and biomass energy systems are in various stages of development and their applications are steadily increasing. However, one of the sources, which have attracted considerable attention in recent years, is the biofuels such as bioethanol and biodiesel. Biofuels can play an essential part in reaching the target to replace petroleum based transportation fuels and in reducing CO₂ emissions, in environmental and economic sustainability are considered carefully (Yuan et al., 2008).

First generations of biofuels, which have attained economic levels of production, have been mainly extracted from food, oil crops and animal fats using conventional technology (Nigam and Singh, 2011). Second generation of biofuels have the potential to use waste residues and make use of waste land thereby promoting rural development and improve the economic conditions of developing countries. The most promising second generation biofuel is biodiesel from algae which is capable of using CO₂ and sunlight to produce a variety of organic molecules, particularly, carbohydrates and lipids. These photosynthetic organisms are known to produce high biomass yields with high oil content which can be cultivated in fresh water or wastewater (Hannon et al., 2010). Another advantage of algae is their ability
to tolerate and adapt to a variety of environmental and nutritional conditions. The most positive impact is the utilization of atmospheric CO$_2$ which can have a significant benefit in the context of global warming. However, the water demand for algae is as high as 11-13 million liters/ha/day for cultivation in open pond (Chinnaswamy, 2010). Their ability to grow in fresh water, municipal, industrial wastewaters and sea water not only overcomes this hurdle but also provides treated wastewater for other uses.

Unlike other sources of biofuels, algae have the capability to produce different types of biofuels. Considering the advantages of algae as a biofuel the present work investigated their effectiveness in CO$_2$ sequestration from flue gases in an sequential photobioreactors. This paper discusses the effectiveness of fresh water algae *Chlorococcum* sp for CO$_2$ sequestration from flue gases in a laboratory scale sequential photoreactor with potential to produce biodiesel.

**MATERIALS AND METHODS**

**Algal feedstock**

The algal culture was isolated from an agricultural runoff using the medium described by Bold’s Basal (Illaversi et al., 2010). Cultures were routinely checked for purity by microscopic examination and plating. The pure culture of algae was identified as *Chlorococcum* sp. (KC49075) by 18S rDNA technique.

**Experimental**

Algal cultivation was done in photoreactors consisting of 2L borosilicate glass bottles fitted with rubber stopper. Flue gases mixed with air get desired concentration and bubbled through fine diffuser. The schematic of the experimental set up is shown in Figure 1. The flow rate (20 ml/min) of gas was measured using a rotameter. The photoreactors were irradiated using standard fluorescent lamps (40 w) placed on both the sides. The excess gas was discharged through an outlet tube. The inlet and outlet gas samples were sampled at regular intervals and analyzed for CO$_2$. The algal samples were collected from an outlet at regular intervals and analyzed for various parameters.

**Analytical methods**

**Algal biomass**

The concentration of algal biomass was measured by measuring the optical density of the algal suspension at 680 nm wave length in a UV-visible spectrophotometer (Thermo Electron Corporation Type UV1, England). The dry weight of algae was estimated from a standard graph. Alkalinity of the suspension was measured as per standard procedures (APHA, 1998).

**Fatty acids estimation**

Algal cells were harvested by centrifugation (10000 rpm) for 10 min. The cell pellets separated from the supernatant were washed with distilled water and dried. Fifty mg of dried algal biomass was taken in 15 ml of test tube, 1.6 ml of double distilled water, 4 ml methanol and 2 ml of chloroform were added and mixed thoroughly for 30 S. Thereafter, an additional 2 ml of chloroform and 2 ml of double distilled water were added and solution was mixed for 30 S. Following this, the mixture was centrifuged, at 5000 rpm for 10 min. The upper layer decanted and the lower chloroform layer
containing the extracted lipids was collected in another test tube. The extraction procedure was repeated again with the residual pellet and both the chloroform extracts were mixed to gather and evaporated till dryness. The dried total lipids were measured gravimetrically and lipid content was calculated as percentage of algal biomass.

RESULTS AND DISCUSSION
Since the objective of the study was to evaluate the CO_{2} from flue gases and its sequestration potential of the isolated fresh water algae the growth profile was measured at different CO_{2} concentrations.

Flue gas composition
Combustion of fossil fuel such as coal, oil and gas is the largest CO_{2} emitter globally. Flue gases emitted by refineries mostly contain nitrogen, CO_{2}, oxygen and water vapor. It also has minor amount of CO, SO_{2}, NO_{x} and particulate matter. CPCL, Chennai has supplied flue gas of composition as given in Table 1. The flue gases have 12\% CO_{2} along with NO_{2}, SO_{2} and O_{2}.

Table 1. Flue gas composition on dry basis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gases</th>
<th>Volume,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CO_{2}</td>
<td>12-15</td>
</tr>
<tr>
<td>2</td>
<td>O_{2}</td>
<td>2-5</td>
</tr>
<tr>
<td>3</td>
<td>N_{2}</td>
<td>80-85</td>
</tr>
<tr>
<td>4</td>
<td>CO</td>
<td>500*</td>
</tr>
<tr>
<td>5</td>
<td>SO_{2}</td>
<td>500-800*</td>
</tr>
<tr>
<td>6</td>
<td>NO_{x}</td>
<td>100-300*</td>
</tr>
</tbody>
</table>

*Expressed as mg/NM^{3}

Growth of fresh water Algae
The growth profile of the fresh water algae at two different CO_{2} concentrations from flue gas is shown in Figure 2. At both the concentrations, the growth increased steadily with time reaching a steady state after about 3 days. With high CO_{2} concentration (1070 ppm), there was a sharp increase in growth from 3 days till and stationary stage was reached after 10 days. Similar increase in Chlorella vulgaris growth with increasing CO_{2} concentration has been reported by Zeng et al. (2012). The lower growth rate of algae at low CO_{2} concentration may be attributed to insufficient CO_{2} supply. This is further confirmed from the CO_{2} removal efficiency at both the CO_{2} concentrations shown in Figure 3. The fresh water algae were observed to be more efficient at higher CO_{2} concentration. The maximum removal efficiency increased from 40 \% to 65\% as CO_{2} concentration increased from 500 to 1070 ppm. It was also observed that the trends of CO_{2} removal and growth were similar. Weissman and Tillett (1992) reported that microalgae could convert up to 99\% of CO_{2} in solution.
Since CO\textsubscript{2} is a weakly acidic gas, it affects the alkalinity of the solution. As seen from Figure 4, it was observed that the alkalinity increased from 180 to 560 mg/l during the growth of algae at both the CO\textsubscript{2} concentrations. However, the pH of the solution remained constant throughout the growth at both CO\textsubscript{2} concentrations. Rangarao et al. (2007) have observed that bubbling of CO\textsubscript{2} continuously resulted in decrease in pH of culture solution thereby fall in cell density.

**Figure 4:** Variation in alkalinity during growth of fresh water algae

**Lipid content of fresh water algae**

The fresh water alga was not only evaluated for its CO\textsubscript{2} sequestration potential based on its growth, but also for its potential use as a feed stock for biodiesel. This was determined from the lipid content of the algal cell given in Figure 5. It was observed that as the cell growth increased with the time, its lipid content was also increased, with a maximum of 38% in 5 days. However, this is much lower compared with the 20-40% lipid content reported for same algal strains (Hu et al. 2008). Liu et al. (2008) reported that total lipid contents representing 20-50% of the dry weight were found to be quite common. Go et al. (2012) reported 12.2 mg/g/day oil productivity in marine algae *Tetraselmis suecica*.

**Figure 5:** Fatty acid content during the growth of algae on flue gases

**Fatty acid profile of fresh water algae on flue gas**

The fatty acid profile of *Chlorococcum* sp. cultivated under flue gas showed similar pattern regardless of the CO\textsubscript{2} concentration from flue gas. Among the identified fatty acids, the proportion of C18:2, C18:3, C20:2, C20:2A were identified (Table 2) which ranged from 0.310 to 4.171% of the total identified fatty acids—polyunsaturated fatty acids (PUFA). On the day 6. In particular C18:2 and C18:3, which is dominant fatty acid. Although the attention was given for PUFA, there was a presence of the unidentified fatty acids over the total fatty acids. For the successful production of PUFA from microalgal biomass, the fatty acid composition of the total lipids is equally important as amount of lipid produced (Ho et al., 2010). An important factor that influence, the lipid content and the fatty acid composition during cultivation of microalgae is the growth phase (Mansour et al. 2003). In *Chlorococcum* sp., C18:2 and C18:3 was the main component and
therefore algae can be a good candidate for the production of high quality PUFA and biodiesel. 

Table 2. Fatty acid profile of fresh water algae grown on flue gases

<table>
<thead>
<tr>
<th>Fatty acid name</th>
<th>Lipid number</th>
<th>Fatty acid Concentration µg/mg of dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoelidic acid ME</td>
<td>C18:2T</td>
<td>0.00</td>
</tr>
<tr>
<td>Linoleic ME</td>
<td>C18:2C</td>
<td>41.71</td>
</tr>
<tr>
<td>Linolenic ME</td>
<td>C18:3</td>
<td>10.50</td>
</tr>
<tr>
<td>g-Linolenic ME</td>
<td>C18:3c</td>
<td>0.00</td>
</tr>
<tr>
<td>11,14-Eicosatrienoate ME</td>
<td>C20:2</td>
<td>6.84</td>
</tr>
<tr>
<td>Arachidonic ME</td>
<td>C20:4</td>
<td>0.00</td>
</tr>
<tr>
<td>8,11,14-Eicosatrienoate</td>
<td>C20:3c</td>
<td>0.00</td>
</tr>
<tr>
<td>5,7,11,14,17-Eicosapentanoic Acid ME</td>
<td>C20:5</td>
<td>0.00</td>
</tr>
<tr>
<td>11,14,17-Eicosatrienoate ME</td>
<td>C20:2</td>
<td>3.10</td>
</tr>
<tr>
<td>4,7,10,13,15,19-Decosahexanoic acid</td>
<td>C22:6</td>
<td>0.00</td>
</tr>
</tbody>
</table>

CONCLUSION

A biological bioreactor was designed to remove CO₂ from flue gases using algae Chlorococccum sp with high growth rate and lipid content in presence of flue gases. This strain could grow at 500 ppm and 1070 ppm of CO₂ from flue gases with 30-35% of fatty acid production. Among the fatty acid specially PUFA- C18:2 and C18:3 were prominent in presence of flue gases. Therefore the present results suggests that Chlorococccum sp is a suitable strain for mitigation of CO₂ from flue gases producing PUFA and potential candidate for applications producing high value byproducts.

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