Spirulina Cultivation Using Biogas CO_2 as the Carbon Source: Preliminary Study on Biomass Growth and Productivity

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Abstract. Anthropogenic activities are causing a rapid increase in global carbon dioxide (CO_2) emissions, which significantly contribute to global warming. Microalgae can be a sustainable solution for simultaneous wastewater treatment and sequestering CO_2 through photosynthesis. The current study reports a comparative evaluation of *Spirulina* sp. microalgal biomass growth and lipid productivity during its cultivation supplied with air and biogas from an anaerobic digester. It was observed that there was a 4-fold increase in biomass productivity in the reactor sparged with biogas compared to air supply. The reactor sparged with biogas showed a significant increase in lipid content. This increase in biomass productivity could be attributed to the increased availability of CO_2, favoring algal growth.

Keywords. Biogas, Biomass productivity, CO_2 sequestration, Lipid content, *Spirulina*

1 Introduction

Anaerobic digestion presents an economically viable and environmentally sustainable approach to organic waste management. Also, it plays a significant role in satisfying global renewable energy demands through biogas production [1]. Conventionally, the biogas generated from anaerobic digestors is used for heat/electricity production due to its methane concentration (55-65%). However, it also contains significant amounts of other gases, such as carbon dioxide (30-40%), nitrogen (0-3%), water vapor (5-10%), oxygen (0-1%), and hydrogen sulfide (0-10,000 ppmv) [2]. For the commercial utilization of biogas in combustion engines or natural gas grids, the biogas needs to undergo partial or complete purification and the high levels of CO_2 in biogas result in a decrease in its heating value. When used in internal combustion engines, this elevated CO_2 concentration leads to increased emissions of carbon monoxide and hydrocarbons [3]. The national or regional standards regulations stipulate that methane content should be $\geq$ 90-95%, carbon dioxide content of $\leq$ 2-4%, and minimal traces of oxygen and hydrogen sulfide in the upgraded biomethane [4]. Biogas purification using physio-chemical technologies such as chemical scrubbing and adsorption requires significant chemical and energy consumption, adversely affecting the economic and environmental sustainability of the process [5]. Moreover, the carbon dioxide that was separated during the upgrading process is emitted into the atmosphere. CO_2 accounts for around 68% of the total atmospheric Green House Gas (GHG) emissions [6]. The continuous increase in its atmospheric concentration is contributing to global warming. Hence, there is an increased interest in alternative technologies that minimize carbon emissions.

Microalgae have recently gained significant attention due to their remarkable ability to fix CO_2 through biomass production and their low operational costs and environmental consequences [7]. Microalgae have a greater CO_2 fixation rate than terrestrial plants, and the CO_2 could be effectively utilized for increased biomass productivity [8]. It was reported that 1 kg of algae could fix approximately 1.83 kg of CO_2 [9]. The produced biomass during CO_2 sequestration can be converted into various value-added products such as biodiesel [10], bioethanol [11], and biohydrogen [12]. Microalgae perform photosynthesis in the presence of light and uptakes CO_2 for their growth. Microalgal biomass is also carbon negative and considered as the third-generation biofuel feedstock [13]. Integrating biogas upgrading using microalgae with simultaneous wastewater treatment can increase the economic and environmental sustainability of the process. The liquid digestate from the anaerobic digesters or the domestic wastewater could be used as a source of nutrients for microalgal growth [14]. For instance, Posadas *et. al*., [15] have employed microalgae for simultaneous biogas upgrading and nutrient removal from a digester centrate in an outdoor high-rate algal pond. Also, it was reported that compared with air sparing, biogas sparging could significantly increase biomass productivity because of high CO_2 availability [16]. The atmospheric level of CO_2 is very low (0.0387% (v/v)) and is insufficient to achieve a high

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rate microalgal biorefinery [17]. The carbon constitutes approximately 60% of the microalgal dry weight, thereby accounting for a significant portion of the associated costs with it during microalgal cultivation [18]. Iamtham *et. al*., [19] have reported a carbon cost saving of 47.6% by utilizing flue gas CO_2 for microalgal cultivation. Hence, employing biogas CO_2 as the carbon source for microalgal cultivation reduces its costs and simultaneously results in biogas with high methane content having a high heating value. Also, several economic value products can be synthesized from the produced algal biomass. Therefore, leveraging biogas as a carbon source for algae cultivation offers significant advantages and holds great potential in sustainable and efficient algae production systems. The present study reports preliminary investigation studies on the comparative microalgal biomass productivity in open-air photobioreactors sparged with air supply and biogas from an anaerobic digester.

2 Materials and methods

2.1 Seed culture

The microalgae used in this study is the *Spirulina* species. The culture was collected from a commercial farm near Hyderabad city, Telangana. The obtained seed culture was transferred to a 2 L conical flask with nutrient media and an air supply of 3 L/min. Table 1 shows the nutrient media composition used in this study.

Table 1. Media composition.

Chemical	Quantity
$NaHCO_3$	8.0 g/L
NaCl	5.0 g/L
Urea	0.2 g/L
K_2SO_4	0.5 g/L
$MgSO_4$	0.16 g/L
H_3PO_4	0.052 mL/L
$FeSO_4^*$	0.05 mL/L
Seed Culture	1.0 mL/L

*- $FeSO_4$- 10 g of $FeSO_4$ was dissolved in 100 mL of DI water, and 10 mL of was acid was added to $FeSO_4$

2.2 Experimental setup

Two duplicate sets of identical glass reactors were fabricated, with dimensions measuring 37 x 33.5 x 25 cm. The total volume of reactors was 30.99 liters with a working volume of 25 liters. Figure 1 (A) and Figure 1 (B) show the experimental reactor setups sparged with air and biogas, respectively. The air supply was provided through sparging, using aerators. The reactors with air supply were considered as the control to monitor *Spirulina* sp. growth under normal conditions.

Similarly, other reactors were sparged with biogas during day time and with air during night time. Diffusers with a surface area of 81.0732 cm^2 were used to distribute air and biogas in the reactors. The biogas flow rate of 0.8 L/min was maintained throughout the study period, while the reactors sparged with air were kept at an airflow rate of 1.5 L/min. The reactors were covered with transparent acrylic sheets to prevent dust or debris deposition. The reactors were kept in open-air conditions under natural sunlight. The average day time during the study period was around 13 h 30 min.

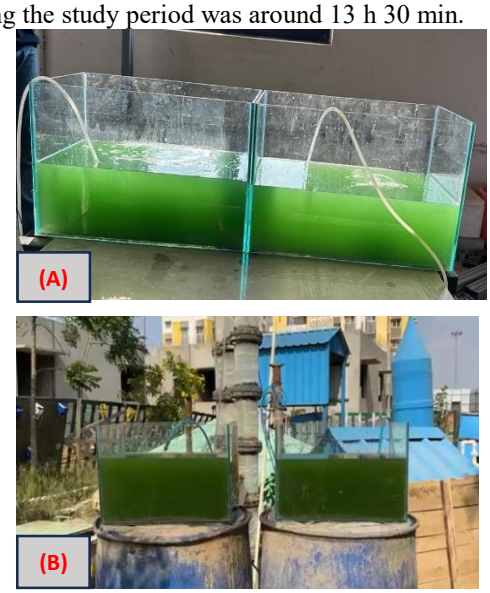

Fig. 1. Microalgal reactor setup used in the study, A- Reactors with aeration, B- Reactors with biogas sparging

2.3 Microalgal growth determination

The microalgal samples were collected daily from all the reactors and analyzed for optical density and chlorophyll content. The optical density was measured at 685 nm wavelength using a UV-Visible spectrophotometer (Make: LAB INDIA, Model: 3000+). The chlorophyll was estimated using method 10, 200 H of the *Standard Methods for Water and Wastewater Examination* [20]. The concentrations of chlorophyll pigments a, b, and c were estimated using the following equations (1-4). Total chlorophyll is calculated by the summation of pigment a, b, and c concentrations.

$$Chl_a\left(\frac{mg}{L}\right) = 11.58 \times (OD_{664}) - 1.54 \times (OD_{647}) - 0.08 \times (OD_{630}) \quad (1)$$

$$Chl_b\left(\frac{mg}{L}\right) = 21.03 \times (OD_{647}) - 5.43 \times (OD_{664}) - 2.66 \times (OD_{630}) \quad (2)$$

$$Chl_c\left(\frac{mg}{L}\right) = 24.52 \times (OD_{630}) - 7.60 \times (OD_{647}) - 1.67 \times (OD_{664}) \quad (3)$$

$$Total\ chlorophyll = Chl_a + Chl_b + Chl_c \quad (4)$$

The correlation between optical density versus dry biomass weight was used to estimate biomass growth. The microalgal biomass productivity (g/L/day) and specific growth rate (1/day) were determined using equations (5) and (6), respectively. N_t and N_i represent the biomass concentration corresponding to the time T_t and $T_{i,}$, respectively. N_m represents the maximum biomass concentration, whereas N_0 is the initial biomass concentration.

$$Biomass\ productivity = \frac{N_t - N_i}{T_t - T_i} \quad (5)$$

$$Specific\ growth\ rate = \frac{\ln(N_m/N_0)}{T_m - T_0} \qquad (6)$$

2.4 Estimation of Lipid Content

The biomass was collected at the end of the study and centrifuged at 5000 rpm for 15 min. The supernatant was disposed of, and the microalgal biomass was kept for drying at 60 ºC in a hot air oven until a constant weight was achieved. The dried biomass was powdered using a mortar and pestle. The lipid content of the powered biomass was estimated by following the modified Bligh and Dyer method [21]. The lipid content was estimated according to the following equation (7).

$$Lipid\ (\%)_{dry\ basis} = \frac{Total\ oil\ extracted}{Dry\ biomass} \times 100 \qquad (7)$$

3 Results and discussion

The change in the optical density with time for microalgal reactors with air and biogas supply is shown in Figure 2. The figure shows that the reactors sparged with biogas showed a significant growth rate compared to the control reactors with air sparging.

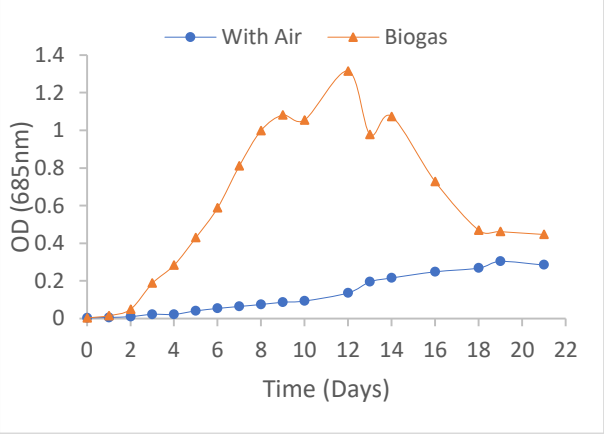

Fig. 2. Change in optical density during the study period.

It is observed from Figure 2 that OD, which is considered the parameter to observe microalgae growth, was found to vary throughout the study period for reactors sparged with biogas. A steep increase in OD was observed during the initial days of reactor operation. OD was found to be increasing up to the 12th day of reactor operation. On saturation of OD on the 12th day of reactor operation, OD declined until the 18th day. Later constant OD was observed up to the 21st day of reactor operation. While there was a rapid change in the OD over the study period in biogas-sparged reactors, air-sparged reactors showed a steady-slow increase in OD was observed until the 18th day of reactor operation. Further, the reactor OD was stable until the 21st day of the reactor operation. Change in the total chlorophyll over the reactor operation period is presented in Figure 3. The overall chlorophyll content was found to be higher in the reactors sparged with biogas throughout the reactor operation period when compared to that of reactors sparged with air. However, in biogas-sparged reactors, chlorophyll values increased steadily until the 12th day and further reduced till the 18th day. It can be observed from Figure 2 that chlorophyll contents variation in both the air and biogas-sparged reactors followed the same trend as that of OD. From Figure 2 and 3, it can be observed that there is a decrease in biomass growth after the 12th day in biogas sparged reactors. This decrease in OD and chlorophyll values could be attributed to a higher biomass growth rate, resulting in nutrient exhaustion leading to cell decay. In the current study, the reactors were operated in batch mode, meaning no additional media or substrate was added during reactor operation. In batch cultures, the cells go through four different phases of the growth cycle: lag phase, log or exponential phase, stationary phase, followed by decay or death phase. In the present study, the lag phase is reduced in biogas sparged reactors, and the exponential growth rate is significantly higher than in the control reactors with air supply. The cultures in the biogas sparged reactors reached the saturation phase earlier than the air sparged reactors, resulting in a limitation of the nutrients (nitrogen and phosphorus). After the 12th day, the culture in biogas sparged reactors might have entered the decay phase due to limited nutrient availability.

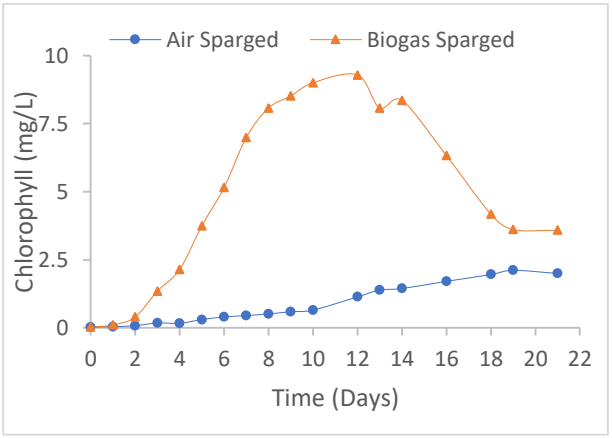

Fig. 3. Change in the Total Chlorophyll during the study period.

Figure 4 shows the correlation plot between dry biomass weight and the optical density. The correlation equation of Y= 476.68x with R^2 of 0.9903 was obtained, depicting a linear relation between dry-weight (Y) and OD (x). The correlation equation was used to estimate the growth rate on dry biomass basis. The maximum biomass productivity of 0.123 g/L/day and specific growth rate of 0.48 per day was observed for the reactor sparged with biogas. Whereas, in control reactors with only air sparging, the maximum biomass productivity and the specific growth rate were observed to be 0.027 g/L/day and 0.23 per day, respectively. Previous studies have reported similar observations for different algal species [22-24]. Jiang *et. al.,* [25] have reported the

biomass productivity of *Nannochloropsis sp*. (sea algae) in the range of 0.161-0.212 g/L. Zhu *et. al.,* [26] have reported the *Spirulina* biomass productivity of 0.195 g/L/day with air supply, which increased to 0.259 g/L/day with increasing the CO_2 concentration to 15%. Also, the specific growth increased from 0.19 per day to 0.26 per day with CO_2 supplementation [26]. In the present study, the biomass productivity rate of reactors sparged with biogas CO_2 was 4-fold higher than that of air-sparged reactors indicating the advantages of biogas application in cultivating *Spirulina* species. In the case of biogas-sparged reactors, biomass productivity was found to be increasing until the 8th day of reactors operation. Further, it decreased, which was negative at the end of the 21st day. This is possibly due to cell death in the reactors, which is also evident from reduced OD and chlorophyll contents. In the case of air-sparged reactors, the biomass productivity was slowly increasing until the 13th day of the reactor run. Further, it slightly decreased until the 18th day of the reactor operation.

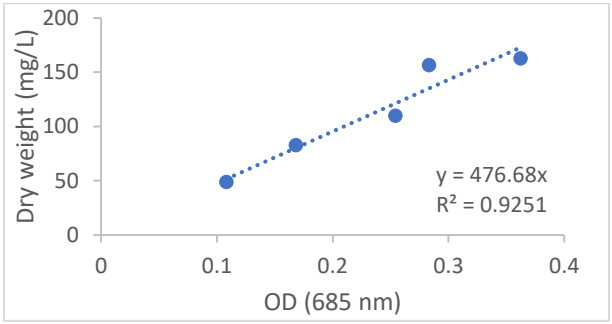

Fig. 4. Correlation between OD and dry-weight.

Microalgae are the photosynthetic microorganisms responsible for more than half of global CO_2 fixation. Microalgae perform photosynthesis in the presence of light by uptaking the dissolved inorganic carbon present in different forms, such as H_2CO_3, HCO_3^-, CO_2, and CO_3^{2-}. This metabolic process is facilitated by the Calvin-Benson cycle, in which the Rubisco enzyme plays a critical role in converting CO_2 into organic compounds. Photosynthesis in microalgae can be categorized into a light-dependent phase and a light-independent or dark phase. In the light-dependent phase, light energy converts NADP+ and ADP into energy-rich NADPH and ATP molecules [27]. The dark phase involves CO_2 fixation and assimilation through the Calvin-Benson cycle by Rubisco enzyme activity, utilizing the NADPH and ATP generated in the light-dependent phase to produce organic compounds like glucose [28]. However, due to its oxygenase activity, Rubisco has a limited affinity for CO_2, resulting in inefficient CO_2 fixation [29]. To overcome this, microalgae employ CO_2 concentrating mechanisms (CCMs) that enhance the localized concentration of CO_2 near Rubisco [30]. CO_2 is one of the limiting substrates in aquatic systems [31]. In the current study, the significant biomass growth rate observed in the photobioreactors supplied with biogas could be attributed to the higher availability of CO_2 for fixation by microalgae. Similar observations have been reported by Miyawaki *et. al.,* [3] during simultaneous biogas purification and wastewater treatment by airlift photobioreactors. They have reported a 70% increase in biomass productivity and lipid content by four times compared with reactors having only an air supply [3]. Jiang *et. al.,* [25] also reported a biomass increase from 0.72 g/L to 2.23 g/L in *Nannochloropsis* sp. cultivated with 15% CO_2 flue gas compared with air supply [25].

The lipids content (dry basis) was estimated using Eq-7. The estimated lipid content for the photobioreactors sparged with air was observed to be 21.2 ± 0.57%. While in the case of photobioreactors sparged with biogas, the lipid content was found to be 28.2 ± 1.41% on a dry basis. The lipid content was found to be 1.4 times more in the photobioreactors sparged with biogas when compared to that of photobioreactors sparged with air. The increase in percentage lipid content was significant. Similar observations have been reported in the literature for different algal species cultivated with CO_2 supply [18,32]. For instance, Saifuddin *et al.,* [33] have reported an increase in lipid productivity from 7.8 mg/L/day to 18.93 mg/L/day by increasing the CO_2 supply from 1% to 15% during *Nannochloropsis* sp. cultivation using CO_2 as the carbon source [33]. El Baky *et. al.,* [34] have also reported that the lipid content was increased from 2.33% to 40.65% by rising CO_2 levels from 0.01% to 12.0%, respectively, during the cultivation of marine microalgae species *Dunaliella salina* [34]. At elevated CO_2 concentrations, the microalgae may prioritize the synthesis of lipids over other components, specifically proteins. This preference is attributed to the mechanism by which the algae capture and utilize extra carbon as an energy reserve to support their growth. Also, increased lipid accumulation in many algal species could be attributed to their adaptive approach to the availability of CO_2 levels within their natural habitat.

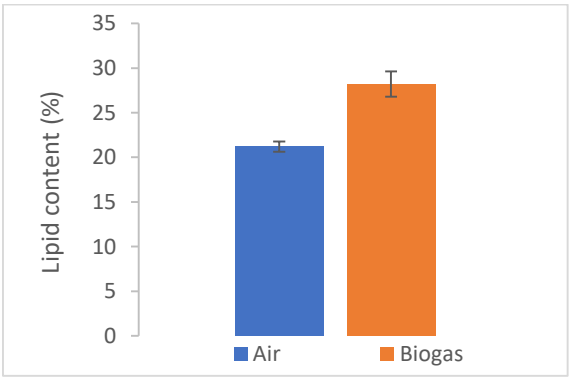

Fig. 5. Lipid contents in the photobioreactor with air and biogas supply.

4 Conclusion

Microalgae has a significant potential for biogas CO_2 uptake. The present study evaluated the growth of *Spirulina* sp. with the supply of biogas CO_2 as the carbon source. This study also investigated biomass

growth and lipid productivity in open-air algal photobioreactors with air supply and biogas sparging. The results demonstrate that the microalgal photobioreactors provided with biogas sparging have shown a 4-fold increase in maximum biomass productivity and higher specific growth rate than reactors with only air supply. The biogas sparging significantly increased lipid content compared to the air sparging. Further studies are needed to optimize the biogas sparging rate and reactor configuration, quantifying the microalgal CO_2 absorption rate and the effect of other environmental factors for an efficient microalgal biorefinery.

Acknowledgment

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