Enrichment of Biogas by Microalgal Scrubbing System and Value added Products Synthesis

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Abstract. Biogas is composed of CH₄ (50-60%) and CO₂ (40-50%), therefore scrubbing (removal) of CO₂ from biogas is imperative as high concentration of it reduces the calorific value and limits its end application. Problems associated with physiochemical process can be alleviated through microalgae as they are efficient in utilizing the CO₂ as its carbon source that can be technically termed as CO₂ sequestration. This study aims to apply the use of a microalgae CO₂ capturing system using Chlorella sorokiniana and Scenedesmus obliquus that have higher photosynthetic capacity and sequestration of carbon dioxide subsequently and transforming into biomass. Chlorella sorokiniana had a total biomass yield of 1.59 g/L and 1.98 g/L for unpurged and purged respectively. Scenedesmus obliquus on the other hand had a better yield over Chlorella sorokiniana with an overall biomass of 1.7 g/L for unpurged and 2.4 g/L under mixotrophic condition. Upon analysis of lipid content in the biomass, the cultures that were grown under mixotrophic condition were found to yield 3.45 and 3.28 fold higher as compared to unpurged sample in Chlorella and Scenedesmus respectively. Under purged conditions, Chlorella sorokiniana had the maximum yield of 5.5 mg/g of vitamin E compared to Scenedesmus obliquus with a comparatively lower yield of 2.32 mg/g. Overall, the increase in the specific growth rate and doubling time indicated that a better adaptability and utilization of carbon source in the purged sample which was provided in the form of 50% citric acid and carbon dioxide. This provides evidence in support of Chlorella sorokiniana for having a higher potential in the area of nutraceuticals. Along with purifying the biogas produced from an anaerobic digester fed with food waste.

Introduction

Anaerobic digestion of wastes is adopted for the production biomethane which can be reused as fuel also mitigation of pollution from the solid wastes especially from agricultural and industrial operations. Biogas constitutes 50-60% of methane and 40-50% of carbon dioxide and has a calorific value which is around 21MJ/m³ of which CO₂ has no heating value [1]. Hence, the total heating value of biogas is directly proportional to the methane concentration. Purification allows for a wider application of biomethane. This is accomplished by biogas up-gradation which is achieved by CO₂ removal which enhances the energy value and provides a consistent gas quality by removing the undesirable gases, such as hydrogen sulphide, carbon dioxide, and moisture, which will be more harmful to be utilized. Though there are physical and chemical methods have been employed for upgradation biological method was profound to be advantageous. One of the finest biological method used for biogas purification is microalgae [2]. Microalgae play a significant role in the biogas upgradation as it has higher efficiency to absorb and tolerate the CO₂ content in the environment. The precedence of microalgae over other methods are environmentally friendly, sustainable and the co-production of high added value materials such lipids, pigments, poly unsaturated fatty acids. In addition, biological
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To provide adequate light and aeration for the appropriate growth of the microalgae, three round bottomed flasks having working volume of 250 mL with a septum for sample collection and aeration were designed (Fig. 1a). The flasks were inoculated with 15% (v/v) algal culture. The aeration in the reactors were provided using an air pump. The experimental sets were categorized into unpurged and purged for both the microalgae (Chlorella sorokiniana and Scenedesmus obliquus). The unpurged microalgal photobioreactors were provided with a defined quantity of citric acid (6 mg/100 mL) whereas the purged microalgal photobioreactors were rendered with 50% of the defined citric acid and CO2 which was derived from the anaerobic digester that was fed with food waste (Fig 1b). The reactors were connected in series and were uniformly aerated with an air pump fitted with 0.45 µm nylon microfilter. Biogas from the anaerobic digester was collected in customized gas columns (Fig 1c), where the gas column was filled with acidified water of pH 2 to avoid pre-solubilisation of CO2 during collection. The anaerobic digester was then connected to the gas column and the congregated gas displaced the water in the column thus occupying the headspace in the column. The pressure was exerted on the

Materials and methods
Microalgae Chlorella sorokiniana (NCIM 5561) and Scenedesmus obliquus (NCIM 5526) were procured from National Collection of Industrial Microorganism (NCIM), Pune, India. BG-11 Media which is contemplated to be the optimal medium for the growth of microalgae was used as a growth medium for the microalgae.

Microalgae can fix much more CO2 than most of the terrestrial and aquatic plants, their efficiency is ten times greater [9]. Microalgae also have a high tolerance to CO2, Scenedesmus sp. and chlorella sp. in particular have shown higher CO2 tolerance. Chlorella sp. could grow successful under 10% CO2 condition and 40% CO2 condition. Scenedesmus sp. was able to grow under 80% CO2 condition. Growth of the two species increased when CO2 was bubbled, variation in the flow rate did not affect the growth [10]. Chlorella sp. has been reported to be better adapted to flue gas as it has shown better growth when exposed to the gases [11]. Scenedesmus sp. has good carbon fixation ability and high biomass productivity. This makes this species suitable for flue gas purification [12]. Flue gas and wastewater may be used as a source of carbon and nutrients for the growth of microalgae with wastewater as the nutrient source and flue gas as the carbon source. This could allow the bioremediation of wastewater along with flue gas purification [13].
C. sorokiniana has been reported to show better growth under mixotrophic conditions with an increase in lipid and protein content. The glucose supplementation helps to increase the lipid content possibly due to the additional energy and material available for biosynthesis. The presence of Acetyl-CoA and NADPH is important for the accumulation of protein and lipid, the presence of glucose enhances these in the medium [14].
S. obliquus has been reported to give increased lipid yield under nitrate deficient conditions, palmitate and oleate constituting the major part of the lipid content which makes it very useful for biodiesel production [15].
Microalgae are a crucial source of nearly all the vitamins such as tocopherol (Vitamin E), ascorbic acid, B1, B2, B6, B12, nicotinic acid and biotin to name a few. They are also rich in macro-minerals and micro-minerals [16]. In this study, biogas produced through anaerobic digestion of food waste was routed through algal bioreactor for removal of CO2 with concomitant growth of microalgae. Further, the enhancement of growth rate under mixotrophic condition and analysis of microbial lipid and Vitamin E accumulation were done. To the best of authors’ knowledge this is the first study report the mixotrophic cultivation of Chlorella sorokiniana and Scenedesmus obliquus and value added product synthesis.

**Fabrication of the Algal Photobioreactor**

To provide adequate light and aeration for the appropriate growth of the microalgae, three round – bottomed flasks having working volume of 250 mL with a septum for sample collection and aeration were designed (Fig. 1a). The flasks were inoculated with 15% (v/v) algal culture. The aeration in the reactors were provided using aeration pump. The experimental sets were categorized into unpurged and purged for both the microalgae (Chlorella sorokiniana and Scenedesmus obliquus). The unpurged microalgal photobioreactors were provided with a defined quantity of citric acid (6 mg/100 mL) whereas the purged microalgal photobioreactors were rendered with 50% of the defined citric acid and CO2 which was derived from the anaerobic digester that was fed with food waste (Fig 1b). The reactors were connected in series and were uniformly aerated with an air pump fitted with 0.45 µm nylon microfilter. Biogas from the anaerobic digester was collected in customized gas columns (Fig 1c), where the gas column was filled with acidified water of pH 2 to avoid pre-solubilisation of CO2 during collection. The anaerobic digester was then connected to the gas column and the congregated gas displaced the water in the column thus occupying the headspace in the column. The pressure was exerted on the
accumulated gas by passing water which oozed out the gas from the column into the reactors. Uniform purging time of 20 min was maintained. The CO₂ dissolves in the media forming carbonic acid Eq. 1 which can be utilized by algae thus subjecting the mixotrophic mode of nutrition.

\[ CO_2 + H_2O \rightarrow H_2CO_3 \]  

(1)

**Fig 1.** (a) Microalgae based photo bioreactors for CO₂ scrubbing; (b) Diagrammatic representation of CO₂ scrubbing; (c) Setup for CO₂ scrubber

**Biomass Analysis (Dry cell weight basis)**

Homogenized algal cell suspension of volume 10 ml was collected from the photobioreactor and optical density (OD) was measured at an equal interval of 12 hours at 750 nm for total duration of 240 hours i.e., 10 days. The samples were centrifuged (5000g, 10 min, 4 °C) and the biomass was separated, which was washed later with 0.85 % (w/v) NaCl solution and dried at 80 ± 5 °C in hot air oven [17]. Furthermore, dry cell weight was calculated using the following formulae:

Chlorella sorokiniana

\[ \text{Dry cell weight (g)} = 7.16 \times OD_{750} \]  

(2)

Scenedesmus obliquus

\[ \text{Dry cell weight (g)} = 8.85 \times OD_{750} \]  

(3)

**Growth Kinetics**

The specific growth rate (\( \mu_{\text{act}} \)) and doubling time (\( t_d \)) of the unpurged and purged samples of Chlorella sorokiniana and Scenedesmus obliquus were calculated with the acquired data of biomass analysis.

\[ \ln \frac{N_t}{N_0} = \mu \times t \]  

(4)

Where \( N_t \) = Concentration of biomass at time ‘t’  
\( N_0 \) = Initial concentration of biomass  
\( k \) = First order growth constant (i.e., specific growth rate (h⁻¹))  
\( t \) = Time (h)

\[ t_d = \frac{0.693}{\mu} \]  

(5)

Where \( t_d \) = Doubling time (h)  
\( \mu \) = Specific Growth Rate (h⁻¹)
2.4 Chlorophyll Estimation
Chlorophylls are believed to be the major pigments that dominate the colour incongruity. Microalgal cell suspension (unpurged and purged) samples of volume 10 ml each was collected for *Chlorella sorokiniana* and *Scenedesmus obliquus* from the photobioreactors during the course of growth study and centrifuged (5000g, 10 min, 4ºC). The pellets were suspended in 3 ml methanol and each samples were sonicated for 5 min at 2 KHz. The optical density was measured at 663 nm and 646 nm for the quantification of the pigments.

Chlorophyll concentration was calculated using the following equation [18],

\[
\text{Chlorophyll a (mg/ml)} = 16.72 \, A_{665} - 9.16A_{652}
\]  

(6)

\[
\text{Chlorophyll b (mg/ml)} = 34.09A_{652} - 15.28A_{665}
\]  

(7)

2.5 Confocal Microscopy
Microalgae consist of chlorophyll and therefore possess the ability of auto-fluorescence. The samples were checked for morphological similarity and average cell size using a confocal microscope. For the sample preparation. The sample was excited at 488 nm and the auto fluorescence was examined. The average cell size of the cells were determined using a confocal microscope.

2.6 Lipid Estimation
The biomass was dried for lipid analysis and the lipid dry weights were determined using Bligh Dyer method [19].

Lipid yield was calculated using the following equation:

\[
\text{Lipid Yield (mg/L)} = \left( \frac{\text{Lipid Dry Weight (mg)}}{\text{Volume of Sample (L)}} \right)
\]  

(8)

2.7 Qualitative Estimation of Vitamin E (Tocopherol) Using HPLC
For the analysis, an Agilent 1260 infinity series equipped with UV detector and Zorbax eclipse C18 analytical column (5 µm, 250X mm) was used. A wavelength of 295 nm was used for Tocopherol detection. Qualitative analysis was done using single point calibration using pure α-Tocopherol and the area under the curve.

3. Results and discussion
3.1 Growth Characterization
*Chlorella sorokiniana* and *Scenedesmus obliquus* were grown in BG11 media with citric acid as the carbon source and sodium nitrate as the nitrogen source. The growth pattern was observed for 10 days with 12 h intervals. The OD readings were taken @750nm. Fig 2 shows the lag, log and stationary phases observed for both the strains. It has been observed from Fig 2 that *Chlorella sorokiniana* was in the lag phase till 48 h after which it transitioned into log phase from 48 hrs. The lag phase continued until 216 h after which the stationary phase began. Light and dark cycle as maintained during the growth. *Scenedesmus obliquus* was observed to have begun with lag phase till 72 h after which it entered into log phase from 72 h and continued until 192 h after which the stationary phase began. The photocycle was provided for 16/8 hours.

![Fig 2. The growth curve of Chlorella sorokiniana and Scenedesmus obliquus](image-url)
The purged culture grown under mixotrophic condition, as it has two carbon sources, one is citric acid (50%) present in the media and the other carbon source is the CO\textsubscript{2} present in the biogas. Fig 3 (a) indicates that the purged sample and the unpurged sample of *Chlorella sorokiniana* shown an increased pattern in the biomass yield over time but there is a surprisingly sharp increase in the dry biomass yield observed after 120 h for the purged sample. Similarly, for *Scenedesmus obliquus* as observed in Fig 3 b, the purged samples grew better than the unpurged indicating that mixotrophic conditions allow better growth than photo heterotrophic conditions. A sharp increase in biomass was also observed in *Scenedesmus obliquus* after 120 h. Thus indicating that the use of citric acid as a carbon source along with the scrubbed CO\textsubscript{2} from the biogas enhanced the growth of the algae. The CO\textsubscript{2} reacts with the water in the algal culture and forms carbonic acid which is used as the inorganic carbon source, while the citric acid acted as the organic carbon source. *Chlorella sorokiniana* had a total biomass yield of 1.59 g/L and 1.98 g/L for unpurged and purged respectively. *Scenedesmus obliquus* on the other had a better yield over *Chlorella sorokiniana* with an overall biomass of 1.7 g/L for unpurged and 2.4 g/L under mixotrophic condition. Tang et al., [20] reported that two similar microalgal strains *Scenedesmus obliquus* SITU-3 and *Chlorella pyrenoidosa* SITU-2 were found to be tolerant while subjecting it 50% CO\textsubscript{2} resulting in a biomass yield of 0.69 g/L, and accumulating even higher biomass of 1.22 g/L when the CO\textsubscript{2} concentration was maintained up to 20%. *Chlorella sorokiniana* along with glucose as its carbon source had a maximum biomass yield of 3.55 g/L under mixotrophic condition [21]. Studies also suggest that the biomass accumulation is enriched under mixotrophic cultivation [22].

### 3.2 Growth Kinetics

The specific growth rate of the unpurged sample ($\mu_{\text{unpurged}}$) of *Chlorella sorokiniana* and *Scenedesmus obliquus* was determined using the first order growth (Eq. 4). From Table 1, it has been observed found that $\mu$ value of 0.37 d\textsuperscript{-1} and 0.36 d\textsuperscript{-1} obtained from *Chlorella sorokiniana* and *Scenedesmus obliquus* respectively, while the purged sample ($\mu_{\text{purged}}$) had a specific growth rate of 0.39 d\textsuperscript{-1} and 0.43 d\textsuperscript{-1} respectively. The doubling time ($t_d$) of the unpurged sample of *Chlorella sorokiniana* was found to be 45 h (1.8 days), whereas the $t_d$ of purged sample was 42.6 h (1.7 days). A slight difference in the doubling time $t_d$ was observed. The $t_d$ of the unpurged and purged samples of *Scenedesmus obliquus* was 46.2 h (1.9 days) and 38.5 h (1.6 days) respectively. Reported studies indicated that *Chlorella sorokiniana* exhibited a maximum specific growth rate of 0.50±0.0\textsuperscript{1} and 0.26±0.005 d\textsuperscript{-1} for *Scenedesmus obliquus* under unpurged conditions [23,24]. In the present study, $\mu$ and $t_d$ of the purged samples of *Scenedesmus obliquus* were found to be higher proving that it is more compatible and highly efficient under mixotrophic cultivation [25]. Overall, the increase in the specific growth rate and doubling time indicated that a better adaptability and utilization of carbon source in the purged sample which was provided in the form of 50% citric acid and carbon dioxide.
Table 1. First order growth kinetic parameters obtained for *Chlorella sorokiniana* and *Scenedesmus obliquus* under unpurged (heterotrophic) and unpurged (mixotrophic) conditions.

| Parameters                  | Units       | *Chlorella sorokiniana* | *Chlorella sorokiniana* | *Scenedesmus obliquus* | *Scenedesmus obliquus* |
|-----------------------------|-------------|-------------------------|-------------------------|------------------------|------------------------|
|                             |             | Heterotrophic cultivation | Mixotrophic cultivation | Heterotrophic cultivation | Mixotrophic cultivation |
| Specific growth rate (µ)    | d⁻¹         | 0.37                    | 0.39                    | 0.36                   | 0.43                   |
| Doubling time (t_d)         | h           | 45                      | 42.6                    | 46.2                   | 38.5                   |
| R²                          |             | 0.93                    | 0.91                    | 0.89                   | 0.94                   |

3.3 Chlorophyll profiling

In comparison to the chlorophyll content of the unpurged and purged samples of *Chlorella sorokiniana* and *Scenedesmus obliquus*, higher chlorophyll content was found in the purged sample which supports for the higher lipid content in the purged sample than the unpurged. Fig 5, represents the Chlorophyll a and Chlorophyll b content. Chlorophyll b content was found to be higher under purged and unpurged conditions for both *Chlorella sorokiniana* and *Scenedesmus obliquus*. In comparison, *Scenedesmus obliquus* had a higher chlorophyll content and therefore it will be favorable for lipid production. Mixotrophic conditions favors chlorophyll enrichment in microalgae [26].

3.4 Lipid Content

The lipid accumulation in the cells is estimated by dry cell weight method. The lipid content in both species *Chlorella sorokiniana* and *Scenedesmus obliquus* was found increasing when there was an increase in biomass. Under mixotrophic conditions, acetyl CoA carboxylase plays an important role in lipid accumulation. Also, nitrogen-deficient conditions were provided which further added to the high lipid accumulation inside the cells. Table 2 indicates the lipid yield of *Chlorella sorokiniana* and *Scenedesmus obliquus* under purged and unpurged conditions. Under mixotrophic condition, a lipid yield of 920±25 mg/l from *Chlorella sorokiniana* and 1180±35 mg/l from *Scenedesmus obliquus* was obtained. There was a 3.5 fold increase of lipid in *Chlorella sorokiniana* and a 3.2 fold increase in *Scenedesmus obliquus* was observed under purged condition over unpurged. The lipid yield under mixotrophic condition was three fold higher than heterotrophic condition as reported by [14, 15].

3.5 Confocal Microscopy

Since microalgae contain chlorophyll and it has the property of auto fluorescence. The morphological structure of the unpurged and purged sample cells are found to be similar and this shows the cells of the purged sample are not in any stress conditions. There is a decrease in the cell size under mixotrophic condition as observed in fig 6. In fig 6 (a), for the unpurged sample of *Chlorella sorokiniana* the maximum cell size was 4.85 µm which was found to be reduced to 4µm under purged condition as seen in fig 6 (b). Similarly, in fig 6 (c), the maximum cell size observed was 4.11 µm for *Scenedesmus obliquus* and a decreased cell size of 3.39 µm under purged condition was observed in fig 6 (d). The purged samples of *Chlorella sorokiniana* had a 15.1% reduced cell size while *Scenedesmus obliquus* cell size was reduced by 18.58%. Due to the increased growth rate observed under mixotrophic condition, the cells utilized the carbon and nitrogen sources rapidly during the log phase which caused the nutrient deficiency at the end of the log phase. Therefore a reduction in the cell size was observed under purged condition due to the nitrogen-deficient environment.
Table 2. Lipid yield of *Chlorella sorokiniana* and *Scenedesmus obliquus* (unpurged and purged)

| Sample          | Lipid dry cell weight (mg) | Lipid yield (mg/l) | Reported yield (mg/L) with pure glucose |
|-----------------|----------------------------|--------------------|----------------------------------------|
| *Chlorella sorokiniana* |                            |                    |                                        |
| Unpurged        | 13±0.8                     | 260±18             | 349±35 [15]                            |
| Purged          | 46±1.3                     | 920±25             |                                        |
| *Scenedesmus obliquus* |                            |                    |                                        |
| Unpurged        | 18±0.5                     | 360±20             | 318.1±10.5 [14]                        |
| Purged          | 59±0.6                     | 1180±35            |                                        |

Fig 4. Chlorophyll a and b (a, b) of *Chlorella sorokiniana* and *Scenedesmus obliquus* (unpurged) respectively; (c, d) Chlorophyll a and b of *Chlorella sorokiniana* and *Scenedesmus obliquus* (purged) respectively.

3.6 Vitamin E Estimation

Vitamin E estimation was carried out using HPLC. Fig 7 (a) specifies the retention time of tocopherol acetate (standard) and it was found between 3-4 minutes. Similar peaks were obtained in fig 7 (a, b) for both the species between 3-4 minutes. Under purged conditions, *Chlorella sorokiniana* had the maximum yield of 5.5 mg/g compared to *Scenedesmus obliquus* which had a comparatively lower yield of 2.32 mg/g. This provides evidence in support of *Chlorella sorokiniana* for having a higher potential in the area of nutraceuticals. Vitamin E production under mixotrophic condition showed a 10 fold increase to that of the autotrophic condition as the reported value for *Chlorella sorokiniana* was around 0.4 mg/g [27], *Scenedesmus sp.* has reported a maximum α-tocopherol production of 0.45 mg/g [28] which is 5 folds lower than that of the estimated value under purged condition. The production of Vitamin E also leads to the full utilization of the process making it more
economical along with the sequestration of CO₂ and hence reducing the need to look out for an alternative for nutraceutical production.

3.7 Biogas Obtained after CO₂ Scrubbing
The ability of CO₂ sequestration by microalgae depends on the growth rate of the microalgae sp. Under the purged condition, the growth rate was observed to be higher in *Scenedesmus obliquus* which ultimately results in enhanced CO₂ scrubbing. *Scenedesmus obliquus* scrubbed 50% CO₂ from the purged biogas whereas *Chlorella sorokiniana* had 23% sequestration capacity. Studies show that *Scenedesmus sp.* has a maximum CO₂ sequestration ability of 80% whereas *Chlorella sp.* has the capacity to scrub up to 40% [10]. Existing physiochemical methods of scrubbing such as lime, membrane adsorbents etc., are not cost-effective and the by-products cannot be reused therefore it is not economical for the long run.

![Fig 5](image.png)

Fig 5. Confocal imaging of *Chlorella sorokiniana* (a) unpurged (b) purged. Similarly, confocal imaging of *Scenedesmus obliquus* (c) unpurged (d) purged (Magnification 40X Scale 5µm).
Fig 6. (a) Vitamin E peak for standard Tocopherol Acetate (100mg/ml) at 295 nm using UV detector at retention time between 3-4 min; (b, c) Vitamin E for *Chlorella sorokiniana* and *Scenedesmus obliquus* at 295 nm using UV detector respectively.

4. Conclusions

The replacement of 100% citric acid in the BG11 medium with 50% citric acid was done and biogas was used to compensate for the rest of the required carbon source. This gave an increase in the yield of the microalgae along with the CO₂ scrubbing of the biogas. The stress conditions provided by reduction in the nitrogen source allowed higher lipid production. The photo-heterotrophic mode of growth in the control set up showed lesser growth in comparison to mixotrophic condition. The mixotrophic mode of growth allowed the algal cells to grow better with less amount of citric acid, making the process more economical. In addition, the vitamin E content was found to be higher in *Chlorella sorokiniana* than *Scenedesmus obliquus* indicating its potential use as a source of nutraceuticals. This study has put forth the concept of biorefinery by mitigation of carbon emission during biogas upgradation. Further, process intensifications are required to adapt these microalgae for higher CO₂ tolerance and enhanced sequestration capacity.

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