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Maximizing Biomass Productivity and CO_2 Biofixation of Microalga, *Scenedesmus* sp. by Using Sodium Hydroxide

Manoranjan Nayak[1,2*], Swagat S. Rath[1], Manikkannan Thirunavoukkarasu[1], Prasanna K. Panda[1], Barada K. Mishra[1], and Rama C. Mohanty[2]

[1]*CSIR - Institute of Minerals and Materials Technology, Bhubaneswar 751 013, India*
[2]*Utkal University, Vani Vihar, Bhubaneswar 751 004, India*

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**Corresponding author*
Phone: +91 94373 65531;
Fax: +91-674-2567160;
E-mail: nayakimmt@gmail.com

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A series of experiments were carried out with three native strains of microalgae to measure growth rates, biomass, and lipid productivities. *Scenedesmus* sp. IMMTCC-6 had better biomass growth rate and higher lipid production. The growth, lipid accumulation, and carbon dioxide (CO_2) consumption rate of *Scenedesmus* sp. IMMTCC-6 were tested under different NaOH concentrations in modified BBM. The algal strain showed the maximum specific growth rate (0.474/day), biomass productivity (110.9 mg $l^{-1}d^{-1}$), and CO_2 consumption rate (208.4 mg $l^{-1}d^{-1}$) with an NaOH concentration of 0.005 M on the 8th day of cultivation. These values were 2.03-, 6.89-, and 6.88-fold more than the algal cultures grown in control conditions (having no NaOH and CO_2). The CO_2 fixing efficiency of the microalga with other alternative carbon sources like Na_2CO_3 and $NaHCO_3$ was also investigated and compared. The optimized experimental parameters at shake-flask scale were implemented for scaling up the process in a self-engineered photobioreactor. A significant increase in lipid accumulation (14.23% to 31.74%) by the algal strain from the logarithmic to stationary phases was obtained. The algal lipids were mainly composed of C_{16}/C_{18} fatty acids, and are desirable for biodiesel production. The study suggests that microalga *Scenedesmus* sp. IMMTCC-6 is an efficient strain for biodiesel production and CO_2 biofixation using stripping solution of NaOH in a cyclic process.

Keywords: Microalgae, biomass, total lipid, fatty acid, photobioreactor

Introduction

Currently, more than 80% of the global energy production is generated from the combustion of fossil fuels [2, 19, 31]. The excessive emission of CO_2 to the atmosphere due to the increase in usage of fossil fuels and rapid industrial revolution has become an environmental concern. The biofixation of CO_2 by microalgae through their photosynthetic activity has been proven to be an efficient and economical method that reduces the carbon content from the environment. The carbon trapped in the biomass mostly gets converted into reduced carbon compounds like lipids and triacylglycerides (TAGs), which are also a potential source of bioenergy [28]. Bioenergy in the form of biodiesel is derived from the transesterification of lipids and TAGs present in algal biomasses. Consequently, the biodiesel produced from microalgae sources has emerged as a carbon neutral fuel [20].

Furthermore, the production of biodiesel from photosynthetic microalgae has several advantages over the other bio-energy producing crops because of the higher photosynthetic efficiency with higher growth rate and lipid content [7, 12, 22]. However, sustainable production of biodiesel at the industrial scale relies upon the high biomass productivity of microalgae [23]. Hence, development of a novel methodology for the production of high lipid-containing biomass has turned out to be the need of the hour.

Carbon is the fundamental element of the living system and it occupies about 50% chemical constituents of microalgae biomass [13]. As CO_2 is the source of carbon for microalgae, the rate of CO_2 uptake and biofixation are the vital factors

for photosynthesis. Microalgae utilize dissolved CO_2 from the aqueous medium, but the presence of dissolved CO_2 beyond certain limits leads to a decrease in the productivity of biomass [4, 5]. Hence, proper scientific management is required for the effective utilization of CO_2 for microalgae cultivation.

A number of microalgae species have been reported to be able to utilize carbonates such as Na_2CO_3 and $NaHCO_3$ for cell growth [6, 10, 35]. Microalgae absorb carbon directly from the dissolved CO_2 or HCO_3^- in the culture medium [10]. The mass cultivation of microalgae grown in open ponds under direct sunlight utilizes atmospheric carbon dioxide as a carbon source during photosynthesis. The major challenges with this mode of algae cultivation is related to the limited availability of CO_2, because of the relatively low atmospheric CO_2 concentration (less than 400 ppm) and relatively low rate of CO_2 transfer from the air to the pond water [27]. The algae growth can be promoted by feeding with exhaust gases (flue gases) from industrial plants, which significantly increases the CO_2 bioavailability for algae and concurrently favors the cleaning of the environment. The CO_2 absorption by microalgae does not get influenced by other exhaust gases (flue gases) constituents. Thus, the flue gas containing CO_2 and water can be directly fed to the algae culture system [25]. However, the major drawback in this system is the poor absorption efficiency of CO_2 in water (*i.e.*, 1.25 g/l at 30°C at 1 atm [32], for which around 80−90% CO_2 returns back to the atmosphere [29]). In open pond cultivation, microalgae take CO_2 from the culture media for photosynthesis during the day time, but emit it back to the atmosphere in the process of respiration during the night [9]. Therefore, it is essential to develop an improved system that can retain dissolved carbon for a longer duration in the aqueous culture medium of microalgae. Moreover, the CO_2 retention inside the culture medium can be enhanced by addition of NaOH in the culture medium.

With this brief introduction, the present work deals with the enhancement of the CO_2 retaining capacity of microalgae culture medium by the addition of NaOH. The NaOH supplemented to the culture medium formed a complex with CO_2, and improved its bioavailability for microalgae. As a result, the growth and biomass productivity of the microalgae cultivated in the presence of NaOH was enhanced. The initial microalgae cultivation experiments were conducted in 500 ml shake flasks, with variable concentrations of NaOH and exogenous supply of CO_2. The biomass productivity and CO_2 biofixation efficiency of the aforementioned system have also been compared with the culture medium supplemented with inorganic carbon sources. Furthermore, the microalgae strains were cultivated in a self-designed photobioreactor to evaluate the scale-up possibilities of the process. In addition to biomass generation, the lipid and fatty acid profiles of the microalgae were also studied.

Materials and Methods

Microalgae Strains and Inoculum Preparation

The microalgae strains *Scenedesmus* sp. IMMTCC-6 (freshwater water alga), *Scenedesmus* sp. IMMTCC-13 (brackish water alga), and *Chlorella* sp. IMMTCC-16 (brackish water alga) used in this study were taken from the Culture Collection Centre of CSIR-Institute of Minerals and Materials Technology, Bhubaneswar, India. The strains were cultivated and maintained in a modified bold basal medium (BBM) [33] at pH 6.6. For inoculum preparation, the microalgae maintained on an agar slants were transferred to 50 ml of liquid media and allowed to grow aerobically. After 7 days of culture, the microalgae cells were harvested by centrifugation at 5,000 rpm for 15 min (REMI Cooling Centrifuge, C-24 BL, India) and resuspended into a 3 L Hoffman flask containing 1,000 ml of culture medium. The algal cells at active log phase were used as inoculum in further experiments.

Culture Conditions and Experimental Setup

The microalgae were cultivated in 1,000 ml conical flasks with 500 ml working volume of BBM. All the culture strains were incubated in an automated culture laboratory maintained at 25 ± 2°C, and the cultures were kept under a photoperiod of 16:8 h light: dark cycle at a light intensity of 60 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent tubes. Light intensity was measured using a lux meter (YORCO lux meter, YSI 606, India). The cultures were hand-shaken two to three times daily to avoid adherence of microalgae to the sides of the culture flasks. The pH of the culture solution was measured with the help of a digital pH meter (Systronics 362, India).

Different concentrations of NaOH, which was used as the CO_2 scavenger, were added to the culture media (BBM). Gaseous CO_2 (99.9%) mixed with ambient air was used to prepare CO_2 concentrations of 10%. Cultures were aerated *via* bubbling from the bottom of the flask with an aeration rate of 100 ml/min (*i.e.*, 0.2 vvm, volume gas per volume medium per minute). Continuous aeration was done for 30 min every day. The above study was further compared with the addition of different types of carbonate salts ($NaHCO_3$ and Na_2CO_3) to the culture medium under similar experimental conditions.

Experimental System with Photobioreactor

A self-engineered rectangular photobioreactor (44 cm long, 24 cm wide, and 25 cm high) with a 12 L working volume was used for microalgal cultivation. The photobioreactor was placed

in a cabinet under room temperature (25°C ± 2) and exposed to continuous cool white fluorescent light of intensity 55 µmol $m^{-2}s^{-1}$ at the surface of the photobioreactor. The photobioreactor was equipped with a stirrer and the stirring rate was maintained at 350 rpm.

The microalgal culture in the photobioreactor was supplied with air containing 10% CO_2 at the rate of 2.4 L/min (*i.e.*, 0.2 vvm, volume gas per volume medium per minute) by bubbling from its bottom. Cultures were aerated continuously for 30 min at an interval of 24 h. The samples were collected from the photobioreactor at a regular interval (24 h) for analysis of biomass production by microalgae.

A stock culture of *Scenedesmus* sp. IMMTCC-6 was cultured in a Hoffman flask with 1,200 ml working volume of BBM. After 7 days of culture, the microalgal cells were pelleted by centrifuging at 5,000 rpm for 15 min (REMI cooling centrifuge, C-24BL, India), resuspended with fresh culture medium, and used as inoculums in the photobioreactor.

The optical density of the culture was measured with proper dilution at 750 nm (OD_{750}) at a 24 h interval, using a UV-Visible spectrophotometer (CECIL, UK) for estimation of the biomass production.

Microalgal Dry Weight Estimation

In order to calculate the dry biomass, microalgal cells were collected by centrifugation at 8,000 rpm for 10 min at 4°C, followed by washing of the algal pellets twice with distilled water and drying at 105°C for 24 h [34]. For determination of biomass concentration, regression equations of the cell density (OD_{750}) and dry weight of culture for the three microalgae [4] were used, and correlations were established as shown in Table 1.

Growth Kinetic Parameters and CO_2 Consumption Rate

The specific growth rate (µ/day) was calculated using the following equation:

$$\mu = \ln (W_1/W_0)/\Delta t \quad (1)$$

where W_1 and W_0 are the biomass concentration (g/l) at the end and at the beginning of a batch run, respectively, and Δt is the cultivation time in days [5].

The biomass productivity, P_B (mg $l^{-1}d^{-1}$) was calculated by using the following equation:

$$P_B = C_B \times 1{,}000 / t \quad (2)$$

where C_B (g/l) is the biomass concentration at the end of the batch run and t is the duration of the cultivation.

The lipid productivity P_L (mg $l^{-1}d^{-1}$) was calculated by using the following equation:

$$P_L = C_L/t \quad (3)$$

where C_L (mg/l) is the concentration of lipid at the end of the batch run and t is the duration of the cultivation.

The lipid yield of microalgae Y (%) was calculated using the following equation:

$$Y = W_L/W_{DA} \quad (4)$$

where W_L is the weight of the total lipid and W_{DA} is the weight of the dry algal biomass.

The CO_2 fixation efficiency was calculated daily in terms of CO_2 consumed by the microalga. Moreover, the carbon dioxide consumption rate (P_{CO_2}, mg $l^{-1}d^{-1}$) was derived by using the following equation: [15]

$$P_{CO_2} = 1.88 \times P_B \quad (5)$$

where P_B denotes the biomass productivity (mg $l^{-1}d^{-1}$)

Lipid and Fatty Acid Analysis

Extraction of total lipid from microalgal biomass was done using a modified method of Bligh and Dyer [1]. The lipids were extracted with a mixture of chloroform and methanol (2:1 (v/v)), and separated into chloroform and aqueous methanol layers by the addition of methanol and water, to give a final solvent ratio 2:2:1 of chloroform: methanol: water. The chloroform layer containing lipid was washed with 1% NaCl solution. It was collected and evaporated to dryness using a rotary evaporator. The lipid content of the algal biomass was determined gravimetrically. The fatty acid composition in algal oil was carried out as per the procedure reported earlier [26].

Statistical Analysis

All the experiments were performed in triplicate, and the results in the study were expressed as the mean ± standard deviation (SD) of the three replicates. Standard deviations have been incorporated in the figures in the form of error bars. The Student's *t*-test was used to evaluate difference between groups of discrete variables. A value of $p < 0.05$ was considered as statistically significant.

Results

Comparison of Biomass Growth and Lipid Content in Microalgae Strains

Initially, a comparative study for the growth and lipid content of the three strains was carried out in shake flasks

Table 1. Regression equation of the cell density.

Name of strains	Regression equation of cell density
Scenedesmus sp. (IMMTCC-6)	Biomass (g/l) = (OD_{750})/3.326 (R^2 = 0.997)
Scenedesmus sp. (IMMTCC-13)	Biomass (g/l) = (OD_{750})/2.4586 (R^2 = 0.995)
Chlorella sp. (IMMTCC-16)	Biomass (g/l) = (OD_{750})/2.1655 (R^2 = 0.998)

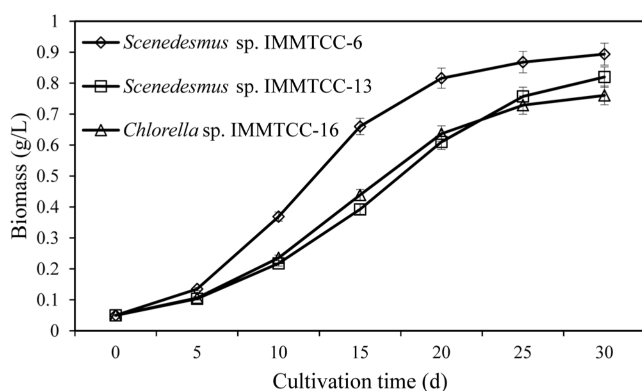


Fig. 1. Biomass variation of *Scenedesmus* sp. IMMTCC-6, *Scenedesmus* sp. IMMTCC-13, and *Chlorella* sp. IMMTCC-16 cultivated in BBM during 30 days of incubation in shake flasks.

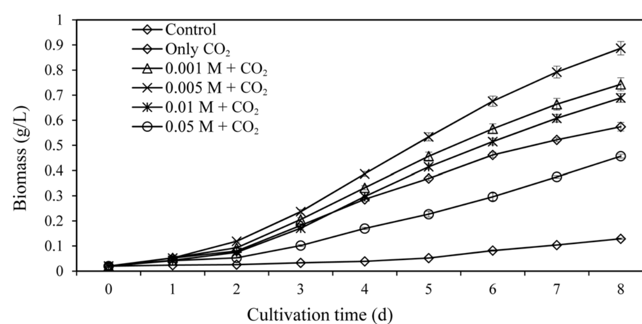


Fig. 2. Effect of the concentration of NaOH on the biomass growth of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM under continuous illumination during 8 days of cultivation time.

for a period of 30 days, without influx of exogenous CO_2 and NaOH to the culture medium. As shown in Fig. 1, the growth of *Scenedesmus* sp. IMMTCC-6 was found to be better than *Scenedesmus* sp. IMMTCC-13 and *Chlorella* sp. IMMTCC-16. The specific growth rate (μ/day) was more in *Scenedesmus* sp. IMMTCC-6 (0.096/day) during 30 days of cultivation time in comparison with *Scenedesmus* sp. IMMTCC-13 (0.093/day) and *Chlorella* sp. IMMTCC-16 (0.091/day). The biomass productivity (P_B) of *Scenedesmus* sp. IMMTCC-6 was approximately 29.79 mg $l^{-1}d^{-1}$, which was more than that produced from *Scenedesmus* sp. IMMTCC-13 (27.33 mg $l^{-1}d^{-1}$) and *Chlorella* sp. IMMTCC-16 (25.35 mg $l^{-1}d^{-1}$) (Table 2).

The biomasses of the microalgae were harvested from the culture media on the 30th day of cultivation, when the microalgae reached the stationary phase of growth and were subjected to total lipid extraction. It was observed that the biomass obtained from *Scenedesmus* sp. IMMTCC-6 had a higher lipid content than *Scenedesmus* sp. IMMTCC-13 and *Chlorella* sp. IMMTCC-16. The lipid yield (Y) of *Scenedesmus* sp. IMMTCC-6 was 24.62%, versus the 13.37% and 18.21% yield in *Scenedesmus* sp. IMMTCC-13 and *Chlorella* sp. IMMTCC-16, respectively. The lipid productivity (P_L) obtained from *Scenedesmus* sp. IMMTCC-6 was 7.33 mg $l^{-1}d^{-1}$, which was more than that obtained from *Scenedesmus* sp. IMMTCC-13 (5.02 mg $l^{-1}d^{-1}$) and *Chlorella* sp. IMMTCC-16 (4.62 mg $l^{-1}d^{-1}$). These results suggest that *Scenedesmus* sp. IMMTCC-6 is a promising strain for biomass growth and lipid accumulation in the present study (Table 2).

Effect of NaOH Concentration on Biomass Growth

The study dealt with the effects of different NaOH concentrations (0.001, 0.005, 0.01, and 0.05 M) in the scrubbing solution on microalgal growth. During 8 days of cultivation in shake flasks, growth parameters such as specific growth rate (μ/day), biomass yield (g/l) and total biomass productivity (mg $l^{-1}d^{-1}$) with CO_2 consumption rate were studied and compared with respect to time. The time course of growth with different concentrations of NaOH under batch mode showed that the biomass increased with NaOH concentration and its optimum was at 0.005 M. On further increase in NaOH concentration, the growth rate decreased (Fig. 2). It was observed that algae are unable to grow with an NaOH concentration of more than 0.05 M (data not shown). The biomass yield (0.887 g/l) on the 8th day of cultivation with 0.005 M NaOH was found to be

Table 2. Specific growth rate, total biomass productivity, lipid productivity, and lipid yield of *Scenedesmus* sp. IMMTCC-6, *Scenedesmus* sp. IMMTCC-13, and *Chlorella* sp. IMMTCC-16 cultivated in BBM in shake flasks for 30 days (stationary phase of the growth).

Name of strain	Specific growth rate	Total biomass productivity	Lipid productivity	Lipid yield
	μ/day	P_B (mg $l^{-1}d^{-1}$)	P_L (mg $l^{-1}d^{-1}$)	Y (%)
IMMTCC-6	0.096	29.79	7.33	24.62
IMMTCC-13	0.093	27.33	5.02	18.37
IMMTCC-16	0.091	25.35	4.62	18.21

Table 3. Performance of biomass production and carbon dioxide utilization of *Scenedesmus* sp. IMMTCC-6 at different NaOH concentrations cultivated in BBM under continuous illumination during 8 days of cultivation time.

NaOH conc.	Specific growth rate μ/day	Biomass yield C_B (g/l)	Total biomass productivity P_B (mg $l^{-1}d^{-1}$)	CO_2 consumption rate P_{CO_2} (mg $l^{-1}d^{-1}$)
Control	0.233	0.129 ± 0.01	16.1 ± 1.2	30.3 ± 2.35
Only CO_2	0.420	0.574 ± 0.02	71.7 ± 2.5	134.9 ± 4.7
0.001 M + CO_2	0.452	0.743 ± 0.03	92.9 ± 3.7	174.6 ± 7.05
0.005 M + CO_2	0.474	0.887 ± 0.03	110.9 ± 3.8	208.4 ± 7.05
0.01 M + CO_2	0.442	0.689 ± 0.02	86.1 ± 2.5	161.9 ± 4.7
0.05 M + CO_2	0.391	0.457 ± 0.01	57.1 ± 1.3	107.4 ± 2.35

the maximum, which is approximately 7-fold more in comparison with that of control (0.129 g/l) media having no NaOH and CO_2. The biomass growth was also much higher than in the media (0.574 g/l) having CO_2 only. It was found that the specific growth rate (0.474 μ/day) and biomass productivity (110.9 mg $l^{-1}d^{-1}$) of the microalgae were maximum in the culture medium supplemented with 0.005 M NaOH (Table 3).

Quantitative analysis for the rate of carbon dioxide consumption (P_{CO_2}) by *Scenedesmus* sp. IMMTCC-6 was done as per Eq. (5) and given in Table 3. A feed of 0.005 M NaOH gave the highest P_{CO_2} of 208.4 mg $l^{-1}d^{-1}$. The results suggested that at the concentration of 0.005 M NaOH, the biomass growth and the rate of CO_2 consumption of *Scenedesmus* sp. IMMTCC-6 were optimal.

Effects of NaOH and Other Inorganic Carbon Sources on Cell Growth and Total Lipid Content

After optimizing the concentrations of NaOH in the previous experiments, a comparative study of culture media with other inorganic carbon sources of $NaHCO_3$ and Na_2CO_3 (all at a concentration of 0.005 M) was done during a 12 day study. It was observed that with NaOH, the lag phase in algal growth was very small and it directly entered the log phase; and on day 9, it followed its early stationary phase (Fig. 3). The control was still in the lag phase. During the same period, other mineral carbon sources were following respective log phases.

The specific growth rate (μ), biomass yield (g/l), and total biomass productivity (mg $l^{-1}d^{-1}$) of the media containing NaOH were 0.438, 0.953, and 79.4, which were higher than other culture media containing inorganic carbon sources ($NaHCO_3$ and Na_2CO_3) (Table 4). The carbon dioxide consumption rates (P_{CO_2}) of *Scenedesmus* sp. IMMTCC-6 have been given in Table 4. Feeding of NaOH gave the highest P_{CO_2} of 149.3 mg $l^{-1}d^{-1}$ on the 12th day of cultivation time.

Our results demonstrated that sodium hydroxide with influx of CO_2 was a superior carbon source for the highest lipid yield of 32.81% and lipid productivity of 26.05 mg $l^{-1}d^{-1}$ (Fig. 4). Lipid yield (16.29%) and lipid productivity (7.43 mg $l^{-1}d^{-1}$) were lowest in the case of microalgae cultivated only with

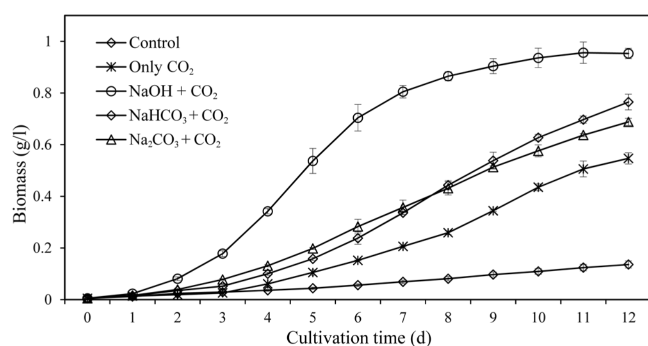


Fig. 3. Biomass growth of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM containing 0.005 M of different carbon sources under continuous illumination during 12 days of cultivation time.

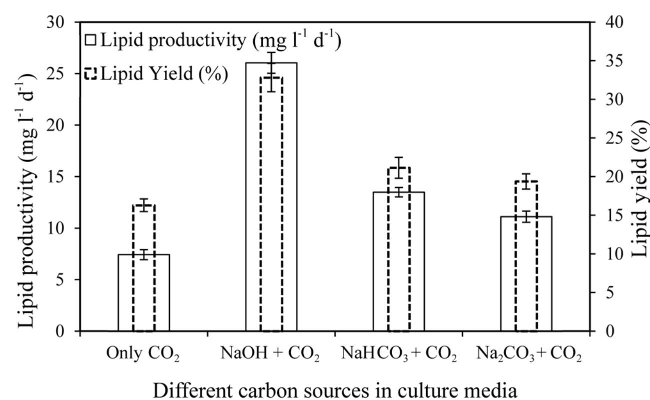


Fig. 4. Lipid yield and lipid productivity of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM containing 0.005 M of different carbon sources under continuous illumination during 12 days of cultivation time.

Table 4. Performance of biomass production and carbon dioxide utilization of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM containing 0.005 M of different carbon sources under continuous illumination during 12 days of cultivation time.

Culture conditions	Specific growth rate μ/day	Biomass yield C_B (g/l)	Total biomass productivity P_B (mg $l^{-1}d^{-1}$)	CO_2 consumption rate P_{CO_2} (mg $l^{-1}d^{-1}$)
Control	0.275	0.136 ± 0.015	11.3 ± 1.2	21.3 ± 2.17
Only CO_2	0.391	0.547 ± 0.019	45.6 ± 1.6	85.7 ± 2.75
NaOH + CO_2	0.438	0.953 ± 0.044	79.4 ± 3.7	149.3 ± 6.36
$NaHCO_3$ + CO_2	0.419	0.765 ± 0.013	63.8 ± 1.1	119.9 ± 1.88
Na_2CO_3 + CO_2	0.41	0.688 ± 0.014	57.3 ± 1.2	107.8 ± 0.02
Photobioreactor[a]	0.244	0.932 ± 0.044	77.7 ± 3.7	146.1 ± 5.51

[a]Photobioreactor containing BBM with 0.005 M NaOH under continuous illumination during 15 days of cultivation time.

CO_2 influx. Microalgae cultivated with sodium bicarbonate and sodium carbonate showed less lipid yield and lipid productivity in comparison with that with sodium hydroxide.

The fatty acid profile under different growth conditions of *Scenedesmus* was studied. The saturated fatty acids (40.61% to 51.15%) were found to be predominant in all culture conditions. It indicates the highest saturated fatty acid content in microalgae cultivated with only CO_2, and the lowest in microalgae cultivated in the presence of NaOH. Following SFAs, MUFAs (29.1 to 35.25) have the higher percentage in comparison with PUFAs (16.94 to 26.1). MUFAs (35.25%) were found to be high in microalgae cultivated in culture media containing NaOH, followed by microalgae cultivated only with CO_2 and microalgae cultivated in the culture media containing Na_2CO_3. It was found to be the least in the case of microalga cultivated in $NaHCO_3$. PUFAs (26.1%) were found to be high in microalgae cultivated in $NaHCO_3$, followed by microalgae cultivated with Na_2CO_3 and NaOH, and were found to be the least in the case of microalgae cultivated in media fed with only CO_2. The overall analysis of fatty acid profiles revealed $C_{16:0}$ (Palmitic acid): 23.8–28.34%; $C_{18:0}$ (Stearic acid): 4.6–8.64%; $C_{18:1}$ (Oleic acid): 19.2–33.51%, $C_{16:1}$ (Palmitoleic acid): 4.27–10.6%; $C_{18:2}$ (Linoleic acid): 8.9–15.22%; and $C_{18:3}$ (Linolenic acid): 2.42–8.4% as the major fatty acids. The fatty acid profile was dominated by saturated and monounsaturated fatty acids, comprising 73.9–83.01% of the entire pool. The quantitative analysis of the lipids generated in the form of FAME profiles revealed that 79–89.27% of the fatty acids belonged to C_{16}-C_{18} type. The culture containing NaOH (89.27%) had the highest C_{16}-C_{18} type of fatty acid among all types of culture conditions.

Cultivation of *Scenedesmus* sp. IMMTCC-6 in Self-Designed Photobioreactor

The cultivation of the microalga was further scaled up in a self-designed photobioreactor at the optimized NaOH concentration (0.005 M). The biomass growth and change

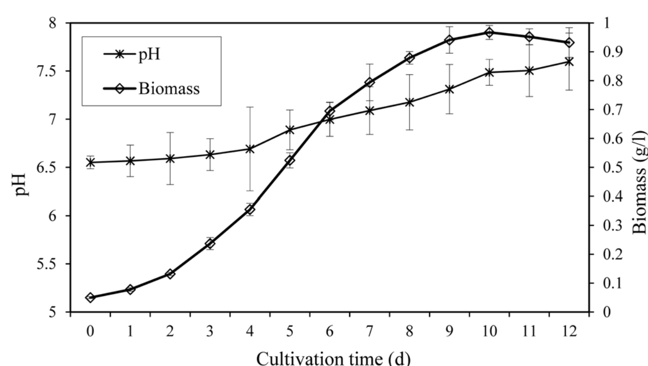


Fig. 5. Biomass growth and pH variations of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM media in photobioreactor containing 0.005 M NaOH under continuous illumination during 12 days cultivation time.

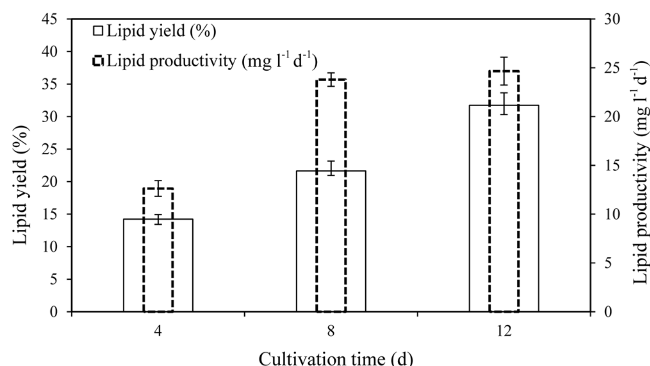


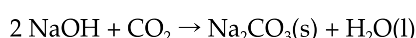
Fig. 6. Variation of lipid yield and lipid productivity of *Scenedesmus* sp. IMMTCC-6 cultivated in BBM in a photobioreactor containing 0.005 M NaOH under continuous illumination during 12 days of cultivation time.

in pH were monitored during a period of 12 days of cultivation (Fig. 5). Biomass growth followed sigmoid type of growth. The specific growth rate, biomass yield, and biomass productivity were calculated to be 0.244/day, 0.932 g/l, and 77.7 mg $l^{-1}d^{-1}$, respectively. P_{CO_2} was found to be 146.1 mg $l^{-1}d^{-1}$ on 12 days of cultivation (Table 4). Microalgal cells from the logarithmic phase, early stationary phase, and stationary phase were collected to measure lipid content. The results indicated that the lipid accumulation in the microalgal cell was associated with the growth phase (Fig. 6). The lipid yield was found to increase from 14.23% in the log phase to 31.74% in the stationary phase of growth. The fatty acid compositions of *Scenedesmus* sp. IMMTCC-6 at the stationary phase in photobioreactor differed from that in shake-flask culture. The percentage of oleic acid ($C_{18:1}$) was found to be significantly increased in PBR (33.51) than that in the shake flask (29.92). It was mainly composed of C_{16}/C_{18} fatty acids, accounting for 88.22% of total fatty acids.

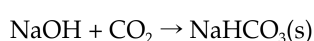
Discussion

For the purpose of biofuel production, microscopic algae have been extensively cultivated owing to their rapid growth rates, high lipid contents, and ability to grow in degraded water bodies. In this study, three primary species with investigated for their biomass and lipid content (*Scenedesmus* sp. IMMTCC-6, *Scenedesmus* sp. IMMTCC-13, and *Chlorella* sp. IMMTCC-16). Each of these species was selected for their distinct morphological characteristics, lipid profiles, and tolerable growth conditions. Of the three algal strains tested, *Scenedesmus* sp. IMMTCC-6 showed better yield in all respects.

The culture media used for the microalgal cultivation did not strip CO_2 effectively. The authors found that the stripping efficiency in the culture media was enhanced significantly in the presence NaOH. CO_2 influx to the culture media, which contain sodium hydroxide, yields sodium carbonate and sodium bicarbonate (equations given below), lowering the solution pH that eventually reaches the pKa of the carbonate system near the neutral range [9]. This condition is suitable for microalgal growth. With increase in time, algae keep taking CO_2 from sodium carbonate/sodium bicarbonate present in the solution favoring the formation of NaOH, which increases the pH in solution. Hence, CO_2 was again fed at an interval of 24 h.



$$2\,NaOH + CO_2 \rightarrow Na_2CO_3(s) + H_2O(l)$$



$$NaOH + CO_2 \rightarrow NaHCO_3(s)$$

The use of sodium hydroxide not only offered enhanced biomass productivity but also met the CO_2 demand of microalgae culture media in a continuous and cyclic process. An optimum concentration of NaOH in the media absorbed and retained dissolved CO_2 and thus facilitated the microalgae growth. Beyond a certain concentration of NaOH, the CO_2 absorption in the media was very high, which had a negative effect on microalgal growth. The biomass production and CO_2 fixation efficiency were best observed in culture media containing 0.005 M NaOH, where the performance of biomass productivity and CO_2 biofixation were approximately seven times compared with control (having no carbon source and no additional influx of CO_2). Previous workers have also quantified the biofixation of carbon dioxide in terms of biomass production in similar way [15–17].

The ultimate targets of microalgal biodiesel production are high lipid yield and high biomass productivity [30]. The percentage of lipid content on the 12th day of cultivation time was measured. It was observed that lipid content (32.81%) and lipid productivity (26.05 mg $l^{-1}d^{-1}$) of media containing NaOH were more in comparison with media containing other carbon sources (Fig. 4). This may be because lipid accumulation was high in media containing NaOH, as it already attained its stationary phase and faced nutrient starvation. Our results are supported by similar studies reporting more lipid accumulation occurring in microalgal biomass during nutrient starvation [12, 22].

Observation on the relationship between the pH of the culture medium and the cultivation time showed that the culture pH increased with increase in cultivation time. Similar observations were shown by Zhao *et al*. [36]. It was also recorded that pH change was directly proportional to the growth rate of microalgae. When the growth rate was more, the alga might have taken more carbon from the carbonate system of the solution, which led to the formation of more OH^-, ultimately resulting in the increasing pH of the solution. After scaling up in the photobioreactor, lipid accumulation was observed to increase with increase in cultivation time and it was mainly composed of C_{16}/C_{18} fatty acids, which are preferable for biodiesel production. Here, the CO_2 sequestered resulted in increased biomass of oil-rich algae *Scenedesmus* sp. IMMTCC-6, and thus offered the opportunity to significantly reduce the cost of its cultivation, which can be put to use for production of biodiesel and CO_2 biofixation. It makes an economical process for carbon recycling of the flue gases emitted from industries and for enhancing algae biomass production as well.

The quality of biodiesel depends mainly on the composition of fatty acid methyl esters. As per the literature, the most common vegetable oils that are used for biodiesel are C_{16} and C_{18} [8], and saturated fatty acids like palmitic acid and stearic acid are the most common fatty acid in biodiesel [21]. Hence, the microalga (*Scenedesmus* sp. IMMTCC-6) studied here is a suitable candidate for biodiesel production. The fatty acid composition of microalga cultivated in media containing different carbon sources differed for different carbon sources. In all cases, the main fatty acid components are C_{16}-C_{18}, which are desirable for biodiesel production [14, 24]. In culture containing NaOH, 29.92% of oleic acid was present, which is a measure of the good quality of the biodiesel [18]. According to the European Standard [11], the linolenic acid content should be less than 12% for a quality biodiesel, which is found to be lower than 12% in all our culture conditions. Microalgae in all culture conditions were found to be composed of palmitic acid (23.8–28.34%), oleic acid (19.2–29.92%), and linoleic acid (8.9–15.22%). The percentage of saturated fatty acid (SFA) was more in comparison with monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). As per the literature, highly saturated fatty acids give an excellent cetane number and oxidative stability to biodiesel [3]. The results showed that NaOH was the suitable source for high growth rate, lipid production, and CO_2 biofixation of *Scenedesmus* sp. IMMTCC-6 among the three tested carbon sources.

Microalgae are currently considered the most feasible biomass in biodiesel production. They can clean smoke stack emissions and use the CO_2 to expedite their growth rate. In the present study, microalga *Scenedesmus* sp. IMMTCC-6 has been found to be capable of higher biomass production and lipid accumulation than *Scenedesmus* sp. IMMTCC-13 and *Chlorella* sp. IMMTCC-16, which can be exploited for efficient biofuel production.

The CO_2 sequestration with microalgae is one of the most promising methods from both cost effective biodiesel production and environmental cleaning points of view. In this study, *Scenedesmus* sp. IMMTCC-6 was cultivated with different NaOH concentrations to examine its abilities of CO_2 biofixation, and production of biomass and lipids. The addition of NaOH (0.005 M) to modified BBM could enhance biomass and lipid productivity in *Scenedesmus* sp. IMMTCC-6. The maximum CO_2 biofixation rate (208.4 mg $l^{-1}d^{-1}$) and biomass concentration (110.9 mg $l^{-1}d^{-1}$) were obtained at 0.005M NaOH for *Scenedesmus* sp. IMMTCC-6. The increase in lipid accumulation (14.23% to 31.74%) by the microalga was observed from the logarithmic growth phase to stationary growth phase. The fatty acid components were mainly composed of C_{16}/C_{18}, which is preferable for biodiesel production. The results suggested that *Scenedesmus* sp. IMMTCC-6 has great potential for CO_2 biofixation and biodiesel production. This strategy presented the potential applicability of NaOH to capture CO_2 and facilitate the biofixation of CO_2 in microalgae in a cyclic process, thereby opening a door for cost-effective biodiesel production from microalgae.

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