Analyzing and optimizing the carbon utilization and lipid yield at different light intensities for *Scenedesmus arcuatus*

Vasumathi K.[1]*, Nadana Raja Vadivu G.[1], Nithiya E.M.[2], Sundar K.[1] and Premalatha M.[2]
1. Department of Biotechnology, Kalasalingam Academy of Research and Education, Virudhunagar, 626126, INDIA
2. Department of Energy and Environment, National Institute of Technology Tiruchirappalli, 620018, INDIA
*mathikrishna@gmail.com

Abstract

Microalgae, the photosynthetic microorganism growing abundantly in marine and aquatic ecosystems, are potential source for biological sequestration of CO_2. The carbon uptake differs in the presence of other nutrients, light intensity etc. The biomass yield of Scenedesmus arcuatus var capitatus was studied based on the Face Centred Central Composite design (FCCD) of Response Surface Methodology (RSM) for nitrate, phosphate and carbonate under different conditions (laboratory, room and sunlight conditions). Various pre-treatments (osmotic shock, autoclaving, microwave and ultrasonication) were employed to find the best method for maximum lipid yield.

The biomass yield reached a maximum of 1 g/L under sunlight conditions of nitrate concentration 500 ppm and carbonate 2000 ppm. The laboratory conditions resulted in a biomass yield of 0.59 g/L at 500 ppm nitrate, 1000 ppm carbonate and 250 ppm phosphate. Under room conditions, the yield was very low (0.11 g/L). Osmotic shock resulted in higher lipid yield than the other pre-treatment methods. The ability of Scenedesmus arcuatus to uptake high carbon under sunlight conditions and to adapt to high light intensity and fluctuations in light intensity concludes that this species is suitable for large-scale open pond cultivation for CO_2 sequestration and production of metabolites.

Keywords: Microalgae, FCCD, *Scenedesmus arcuatus*, sunlight, carbon uptake, lipid extraction.

Introduction

The increasing emission of greenhouse gases has led to issues like global warming and climate change. The international convention like the Kyoto protocol (since the 1990s) is evidence of gaining attention over the reduction of greenhouse gases around the world. CO_2 plays a major role in greenhouse gases.

The atmospheric CO_2 level is found to be increasing at high rates due to industrialization. 54% of the CO_2 emitted from stationary sources is for power generation. India contributes 5% of global CO_2 emissions.

****Author for Correspondence***

Several physicochemical methods like pressure swing adsorption[9], adsorption using diethanolamine (DEA), methyldiethanolamine (MDEA)[25], monoethanolamine (MEA)[1], aqueous sodium glycinate[18] and geological sequestration are available for CO_2 sequestration. The downside of these methods includes the reaction of adsorbents with other acidic compounds in flue gas resulting in undesirable side reactions, surface leaks, CO_2 release due to well blow out or pipeline ruptures of geological/ocean sequestered CO_2, retardation of stability of subsurface water when CO_2 dissolves in subsurface water and also the recovery of amines is expensive as the heat of absorption of formation of the carbamate (carbamate is formed from the reaction of amines with carbon dioxide) is high.[25,33] Also, these methods require the transport of the captured CO_2 to storage sites for geological/ocean storage.

Hence, a new method for utilization of captured CO_2 is required. This could be achieved using microalgae, the photosynthetic microorganism capable of converting captured CO_2 to metabolites (carbohydrates, lipids etc.).[12,16] Hence microalgae strain with high carbon uptake is necessary.

The major factors affecting the growth of microalgae include light[28], light/dark cycle[4] and nutrients (nitrate, phosphate and carbonate), temperature.[6,7,31] Several nutrient media like BB, BG, modified Johnsons, Guillard's f, Zarrouk etc. are available for the growth of microalgae but the suitability in the medium is species-specific. *Scenedesmus arcuatus* is grown commonly in BG11[5,8,34] and medium A.[20]

All the above-mentioned algal growth mediums are composed of multiple nutrients which make the preparation of medium for large scales tedious. Carbon source is essential for photosynthesis; nitrogen is essential for protein synthesis and phosphorus is important for the synthesis of nucleic acids.

Earlier studies have shown the influence of nitrate[8], phosphate[20], carbonate[8], on *Scenedesmus arcuatus*. Hence this study is done with nitrate, phosphate and carbonate only as nutrients. Also under open pond conditions, the sunlight serves as the light source which reduces the expense of providing a light source. Therefore, for given light intensity, the nutrients have to be optimized to maximize the growth.

The present study is on the optimization of nitrate, phosphate and carbonate under laboratory, room and sunlight

conditions by response surface methodology and to find the efficient pre-treatment method for lipid extraction from this species.

Material and Methods

Organism, Conditions and Nutrients: *Scenedesmus arcuatus* available in the Algal Biotechnology Laboratory, DEE was used for this present study.[32] The microalgal species were grown at the light intensity of 160 µE/m^2/s having a light/dark cycle of 12h/12h and at 25ºC in the laboratory conditions. The microalgae were grown with nitrogen (Urea), phosphorus (potassium di-hydrogen) and carbon (potassium hydrogen carbonate) as nutrients.

The reactor used for the growth of microalgae: A flat plate photobioreactor was used in this study with the following dimensions. The surface area to volume ratio of the flat plate photobioreactor was 52.8 m^{-1} and depth 0.06 m. The microalgae were cultivated in 3 replicates.

Analytical method: The growth of microalgae was measured by measuring the optical density of the culture at 440 nm, using a UV/VIS spectrophotometer. The fresh biomass was harvested by centrifugation at 10000 rpm for 3 minutes and air-dried for 48 Hours.

The growth kinetics (specific growth rate) was obtained from the measured optical density:[21]

$$G = [\ln (C_2/C_1)]/t_2 - t_1 \ (day^{-1})$$

where C1 and C2 indicate the cell concentration, t indicates the sampling time. Doubling time (T_d) and the number of doublings (K_d) were also derived from the specific growth rate.

$$\text{Doubling time } (T_d) = (0.6931) / G \ (day)$$

$$\text{Number of doublings } (K_d) = G / (0.6931) \ (day^{-1})$$

Photosynthetic efficiency was calculated from the calorific value of the biomass produced to the incident light intensity. The calorific biomass was measured using the bomb calorimeter. The air-dried samples from the different conditions were analyzed using a bomb calorimeter. The light intensity was measured using the PAR sensor.

Photosynthetic efficiency = (Calorific value of biomass produced (KJ) / (Incident light intensity (KJ))

Optimization studies: Optimization by varying one variable at one time is more time-consuming and also fails to include the interaction between variables. The optimum level of each independent variable and the interaction between variables is achieved by response surface methodology (RSM).[29] This experimental design for nutrient optimization was based on the Face Centred Central Composite Design (FCCD) of RSM and was obtained using design expert. The independent variables studied were nitrogen (Urea, 0-500 ppm), phosphorus (Potassium dihydrogen phosphate, 0-500 ppm) and carbon (Potassium bicarbonate, 0-2000 ppm) (trial version). The experiments were carried out at different light intensities 1) controlled light and temperature (under laboratory conditions 2), diffused sunlight, uncontrolled light and temperature room (room conditions) and direct sunlight conditions (open sunlight conditions). Statistical analysis was performed by a design expert. Data represented are means with standard error.

Effect of cell disruption techniques on lipid yield: The lipid extraction was done by the Bligh Dyer method using a 2:1 ratio of methanol to chloroform. The lipid extraction was done in untreated and pre-treated samples. Four different pretreatment methods such as autoclaving at 120º C for five minutes, microwave treatment at 100º C for 5 minutes, ultrasonication at 20 kHz for 5 minutes and osmotic shock using 10 % NaCl for 48 hours were used to disrupt the algal cells and to find the efficient method for lipid extraction from this species.

Results

Optimization of nutrients at various light intensities: The biomass yield was found to vary with nutrient concentrations. The model equations developed for the experimental variables under laboratory, room and sunlight conditions are given in equations (1) to (3).

Laboratory conditions:

$$R = 0.045991 + 6.24194E\text{-}005 * A + 1.40977E\text{-}003 * B + 4.00590E\text{-}004 * C - 8.74771E\text{-}007 * A * B - 2.99309E\text{-}007 * A * C + 2.45279E\text{-}008 * B * C + 1.08159E\text{-}006 * A^2 - 1.71493E\text{-}006 * B^2 - 1.47663E\text{-}007 * C^2 \quad (1)$$

Room conditions:

$$R = +0.049177 + 1.46136E\text{-}004 * A + 1.19529E\text{-}005 * B - 1.21845E\text{-}005 * C - 3.96818E\text{-}008 * A * B - 1.54734E\text{-}008 * A * C + 1.96382E\text{-}008 * B * C - 1.05204E\text{-}007 * A2 + 9.06581E\text{-}008 * B2 + 1.86699E\text{-}009 * C2 \quad (2)$$

Sunlight conditions:

$$R = +0.59334 - 1.58366E\text{-}004 * A - 1.63072E\text{-}003 * B - 1.39315E\text{-}006 * C - 1.54371E\text{-}007 * A * B + 1.36704E\text{-}007 * A * C - 1.73410E\text{-}007 * B * C + 7.71475E\text{-}007 * A2 + 2.16562E\text{-}006 * B2 + 4.25569E\text{-}008 * C2 \quad (3)$$

Analysis of Variance: Table 2 represents the statistical significance of the equations (1), (2) and (3). The proportion of variation in the response variable is explained by the coefficient of determination (R^2). Since R^2 value is closer to 1, the prediction is better. The value of the adjusted R^2 value is also high. The adjusted and predicted R^2 values are also nearer. This signifies the agreement between the predicted and experimental values. P-value refers to the probability of adding additional terms to the model. The model is significant when P-value is less than 0.05. Since all the

above models show a p-value of less than 0.05, the model is significant.

The actual biomass yield ranged from 0.03 g/L to 1.01 g/L whereas the predicted biomass yield ranged from 0.04 g/L to 1.01 g/L with the highest yield in sunlight conditions. In figure, most of the points lie near the diagonal line which signifies the reasonable agreement between the actual and predicted values of biomass yield under all the above conditions.[15]

Optimization results

Laboratory conditions: The three-dimensional surface plots on the responses obtained from the above experiments are given in figure 1. In figure 1a, the contours show the maximum response at 0 ppm and 500 ppm nitrate whereas phosphate shows the maximum response at 500 ppm for 0 ppm of nitrate and 250 ppm of phosphate for 500 ppm of nitrate. For 500 ppm of nitrate, uptake was found to be optimum at 1000 ppm carbonate (Figure 2b).

From figure 1c, the nitrate and phosphate concentrations were found to be optimum at 1000 ppm and 250 ppm respectively. The maximum biomass yield (0.58 g/L) was obtained at 500 ppm of nitrate, 250 ppm of phosphate and 1000 ppm of carbonate. The least value of biomass yield 0.03 (g/L) was obtained at 0 ppm of nitrate, phosphate and carbonate.

Room Conditions: The three-dimensional surface plots showed a flat response surface and the contours were formed as parallel lines. The nitrate and phosphate increase biomass yield with an increase in the concentration of biomass. But in the case of carbonate, even though 2000 ppm was supplemented, there was a very poor effect on biomass yield and the maximum value was found at 0 ppm.

Sunlight Conditions: The three-dimensional surface plots obtained from the responses are shown in figure 2a, 2b and 2c. Figure 2a explains the increase in biomass yield with increasing nitrate concentration whereas a decrease with phosphate (at 0 ppm of carbonate) but the uptake has shown a positive sign after 400 ppm. In figure 2b, the biomass yield was found to increase with an increase in nitrate and carbonate and the maximum response was obtained at the maximum concentration of both (500 ppm of nitrate and 2000 ppm of carbonate).

Figure 2c demonstrates the effect of carbonate and phosphate at 0 ppm of nitrate. The increasing carbonate concentration has shown an increase in yield whereas phosphate has shown a decrease. Under sunlight conditions of higher light intensity, the maximum biomass yield was obtained at 500 ppm of nitrate, 0 ppm of phosphate and 2000 ppm of carbonate.

Growth characteristics: The growth characteristics under different nutrient concentrations and different light conditions are tabulated in table 3. Under laboratory conditions, the specific growth rate reached a maximum of 0.2868 (d^{-1}) [doubling time = 2.4166 (d), number of doublings = 0.4137 (d^{-1})] at 500 ppm urea, 250 ppm and 1000 ppm. The room condition has shown a decline in specific growth rate and the maximum value obtained was 0.2172(d^{-1}) at 500 ppm urea, 500 ppm and 0 ppm [doubling time = 3.1910(d), number of doublings = 3.1333 (d^{-1})].

The sunlight condition has shown a maximum value of specific growth rate of (0.3834 d^{-1}) [doubling time = 1.8077 (d), number of doublings = 0.5531 (d^{-1})] at 500 ppm urea, 0 ppm and 2000 ppm.

Photosynthetic efficiency: Photosynthetic efficiency is a measure of the amount of incident light that is being utilized by the algae. This is estimated from the amount of biomass produced for given light intensity.

Table 1
FCCD for nitrogen, phosphorus and carbon concentrations.

Variable	Symbol	Unit	Level		
			-1	0	1
Nitrate	A	ppm	0	250	500
Phosphate	B	ppm	0	250	500
Carbonate	C	ppm	0	1000	2000

Table 2
Analysis of variance for quadratic models under laboratory, room and sunlight conditions

Condition	Sum of Squares	df	Mean Square	F Value	P-Value (Prob>F)	Predicted R^2	Adjusted R^2
Laboratory	0.29	9	0.032	52.	0.0002	0.9769	0.9251
Room	6.318E-3	3	2.106E-3	37.2	<0.0001	0.9833	0.9288
Sunlight	0.62	9	0.069	80.71	<0.0001	0.9809	0.9412

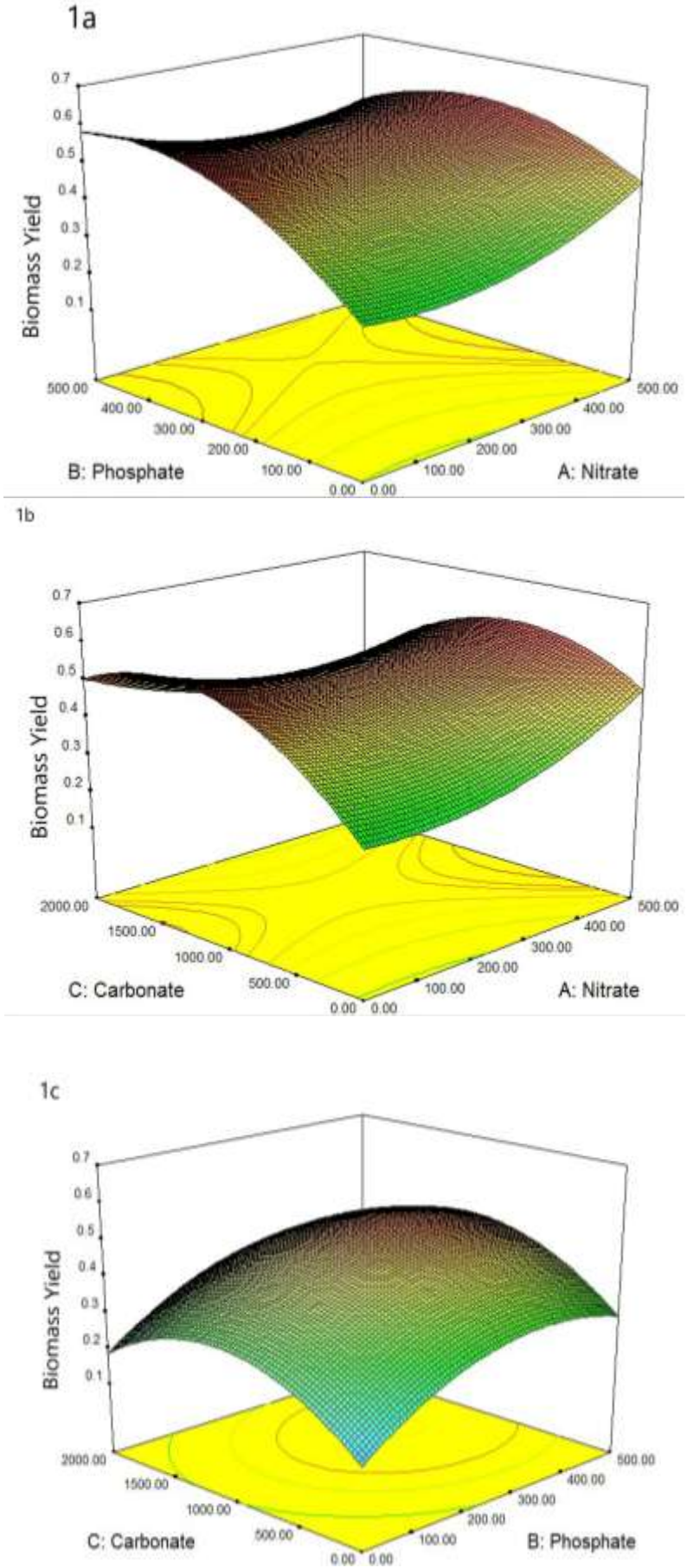

Figure 1: Biomass yield was depicted for the runs at laboratory conditions. The optimized conditions were 1000 ppm of Carbon, 500 ppm of Nitrogen and 500 ppm of Phosphate

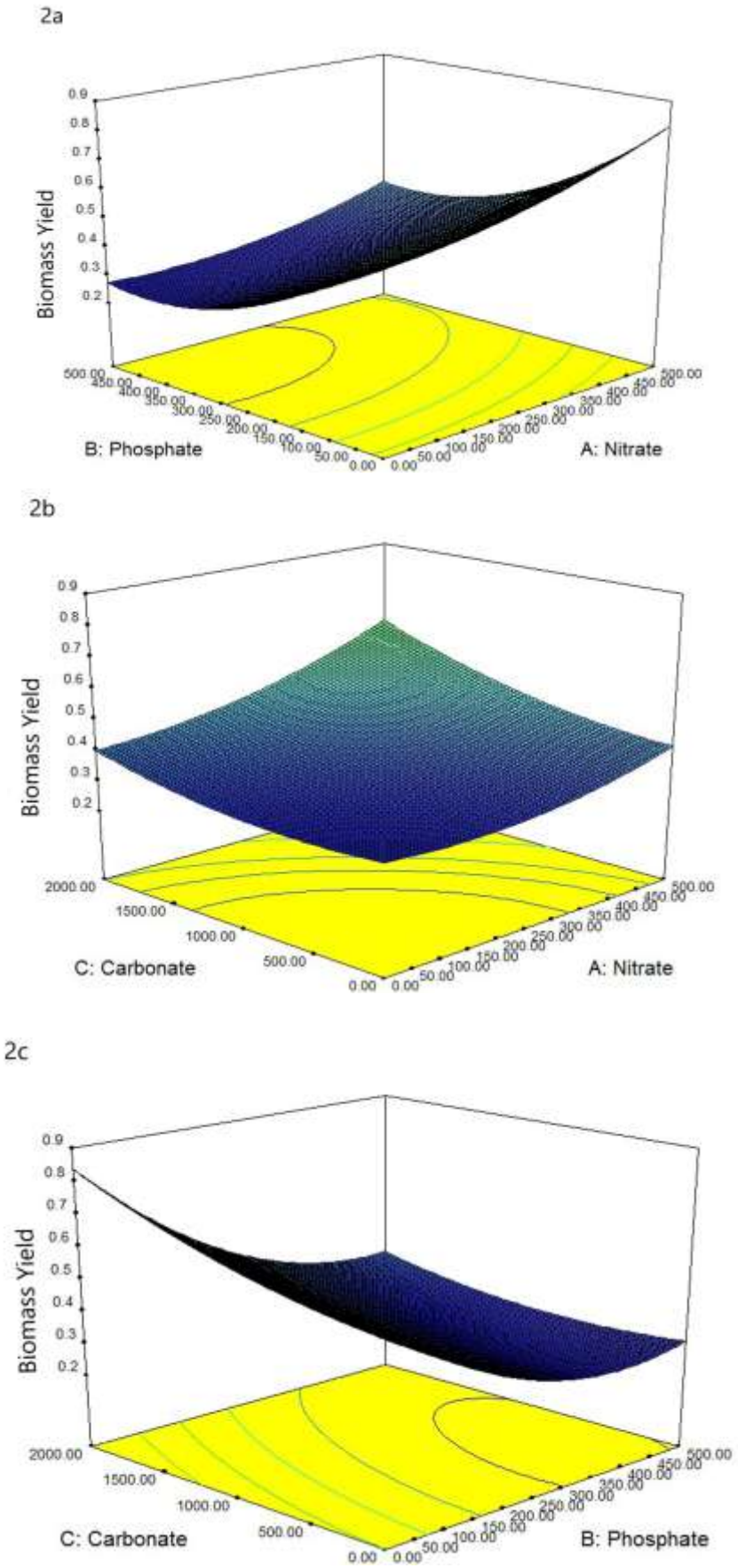

Figure 2: Biomass yield was depicted for the runs in sunlight conditions. The optimized conditions were 2000 ppm of Carbon, 500 ppm of Nitrogen and 250 ppm of Phosphate. This represents the higher carbon uptake under sunlight conditions

Under sunlight conditions, the photons were fluctuating from 400 to 4000 (figure not reported). The average photons available were used for the study. The laboratory conditions have shown a maximum photosynthetic efficiency of 8.75 % at 500 ppm, 250 ppm phosphate and 1000 ppm carbonate whereas room conditions have shown 3.94 % at 500 ppm, 500 ppm phosphate and 0 ppm carbonate. The sunlight conditions resulted in maximum photosynthetic efficiency of 7.85 % at 500 ppm, 0 ppm and 2000 ppm, phosphate and carbonate respectively (Table. 3, Fig.3). This depicts the laboratory conditions that have utilized all the light available in the system.

Under sunlight conditions, the light intensity is more; hence more cells can be used to enhance the photosynthetic efficiency. The carbon uptake was found to be higher under higher sunlight conditions which resulted in higher biomass yield.

Effect of cell disruption techniques on lipid yield: The lipid analysis was performed for the samples collected under sunlight conditions since higher biomass was obtained under sunlight conditions. The results indicate that the carbon utilized was concentrated on lipid metabolism. The increased light intensities may be acting as stress for enhanced lipid yield. Figure 4 shows the lipid yield on different cell disruption techniques. The highest yield was obtained by osmotic shock. Hence osmotic shock is recommended to be used for cell disruption before lipid extraction for *Scenedesmus arcuatus*. The cell nature of the *Scenedesmus* may contain complex substances which are not easily destructed by the other techniques.

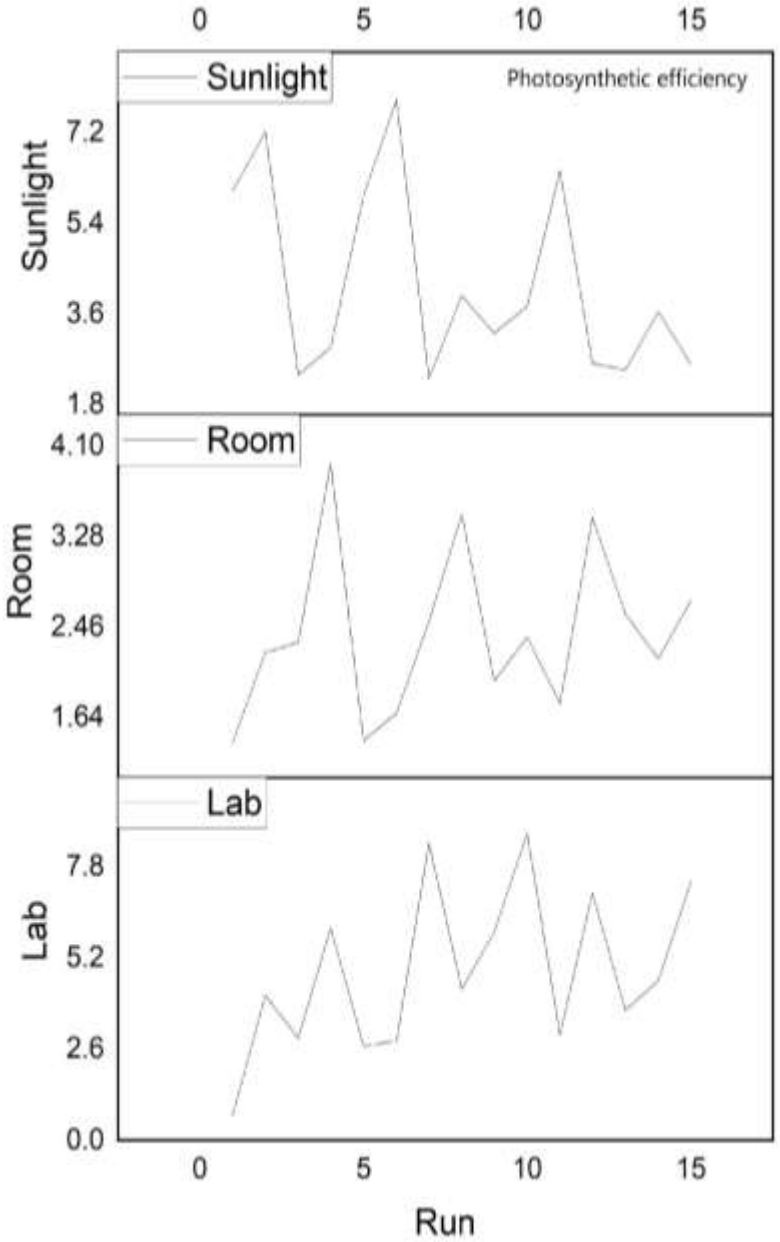

Fig. 3: Photosynthetic efficiency was higher for laboratory conditions compared to others. This may be due to the reduced light intensity and complete utilization of light under laboratory conditions

Table 3
Data (G, T_d, K_d, PE) obtained from various light conditions

Run	Laboratory conditions				Room conditions				Sunlight conditions			
	G (d^{-1})	T_d (d)	K_d (d^{-1})	PE	G (d^{-1})	T_d (d)	K_d (d^{-1})	PE	G (d^{-1})	T_d (d)	K_d (d^{-1})	PE
1	0.0544	12.7311	0.0785	0.69	0.087	7.9666	0.1255	1.41	0.3703	1.8717	0.5342	6.04
2	0.1648	4.2053	0.2377	4.14	0.1031	6.7225	0.148	2.23	0.3154	2.1975	0.4550	7.21
3	0.1209	5.7328	0.1744	2.91	0.1344	5.1569	0.1939	2.32	0.2577	2.6895	0.3718	2.39
4	0.1822	3.8041	0.2628	6.07	0.2172	3.1910	0.3133	3.94	0.2853	2.4293	0.4116	2.94
5	0.1255	5.5191	0.1811	2.69	0.1032	6.7160	0.1488	1.43	0.3288	2.1079	0.4743	5.95
6	0.1328	5.2191	0.1916	2.84	0.0857	8.0875	0.1236	1.68	0.3834	1.8077	0.5531	7.85
7	0.2766	2.5057	0.3990	8.48	0.1848	3.7505	0.2666	2.51	0.1901	3.6459	0.2742	2.32
8	0.1801	3.8484	0.2598	4.32	0.1999	3.4672	0.2884	3.48	0.26805	2.5857	0.3867	3.97
9	0.1901	3.6459	0.2742	5.94	0.0651	10.646	0.0939	1.97	0.2006	3.4551	0.2894	3.22
10	0.2868	2.4166	0.4137	8.75	0.115	6.0269	0.1659	2.37	0.2537	2.7319	0.3660	3.752
11	0.1325	5.2309	0.1911	3.02	0.0555	12.4882	0.08	1.77	0.1901	3.6459	0.2742	6.44
12	0.2692	2.5746	0.3884	7.05	0.2062	3.3612	0.2975	3.45	0.2139	3.2402	0.3086	2.62
13	0.1477	4.6926	0.2131	3.71	0.1414	4.9016	0.2040	2.58	0.2101	3.2989	0.3031	2.49
14	0.1795	3.8612	0.2589	4.56	0.1706	4.0627	0.2461	2.17	0.3316	2.0901	0.4784	3.64
15	0.2566	2.7010	0.3702	7.38	0.1547	4.4802	0.2232	2.70	0.2183	3.1749	0.3149	2.60

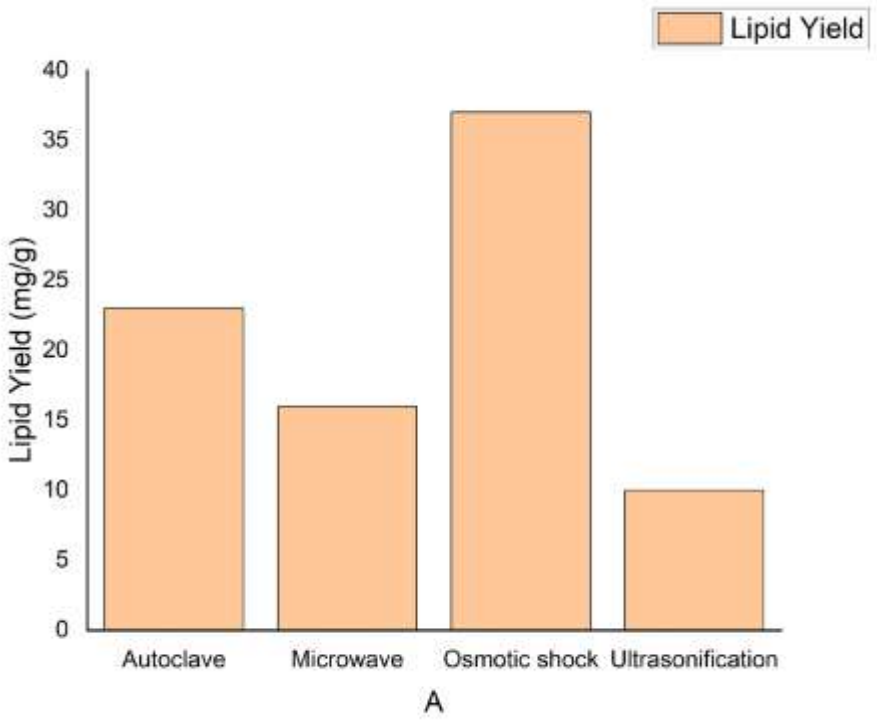

Fig. 4: Lipid yield from different cell disruption techniques. Osmotic shock yielded a higher lipid yield

Discussion

Scenedesmus sp can be able to uptake and grow at 80% CO_2 concentration. *Scenedesmus* and *Chlorella* showed the same good growth rate at 10-50 % CO_2 concentration whereas *Chlorella* was able to sustain at higher light intensities and temperature than *Scenedesmus*.[13] Under high sunlight conditions, the cells become single. Similar results were reported by Hanagata et al.[13] With increasing light intensity, the optimum pH value was also varying for *Chlorella sp*. The cell density has also increased with different light intensities and pH.[10]

Scenedesmus arcuatus has resulted in higher cell densities in potassium bicarbonate rather than sodium bicarbonate. Hence, in the present study, potassium bicarbonate is used. *Scenedesmus sp* can uptake the inorganic carbon source.[23] This combination produced a higher growth rate than the conventionally used medium such as BBM, BG11 etc.[32] Pancha et al[23] a have reported that supplementation of sodium bicarbonate along with BG11 resulted in a similar trend. Under laboratory conditions, the carbonate uptake was optimum at 1000 ppm whereas it reached up to 2000 ppm under sunlight conditions. However, in the case of room conditions, the uptake was poor and the biomass yield (0.11 g/L) was only about one-fifth of that of laboratory condition (0.59 g/L) and one-tenth of that of sunlight (1.01 g/L).[22] This is because of low light intensity. Hence, nutrient addition will enhance growth only if sufficient light intensity is available. The carbon uptake was very slow at room and laboratory conditions whereas in sunlight conditions, the carbon uptake was higher.

In the case of nitrate uptake, sunlight, room and laboratory conditions were excellent at 500 ppm. Under laboratory conditions, nitrate supplementation did not increase biomass yield up to 250 ppm. So, nitrate has to be supplemented at a higher concentration or low instead of an intermediate concentration. The phosphate uptake was poor under sunlight conditions and was optimum at 250 ppm under laboratory conditions whereas it was optimum at 500 ppm under room conditions. So, under sunlight conditions, phosphate supplementation is not needed when carbonate and nitrate sources are supplemented in high concentrations.

The specific growth rate was found to be highest under sunlight conditions (0.3834 d^{-1}). This was found to be less than *Scenedesmus quadricauda* (0.392 d^{-1}) and *Scenedesmus dimorphus* (0.54 d^{-1}) grown in BG11 medium with the light intensity of 2500 to 3500 lux[8] and higher than *Scenedesmus sp*. (0.121 d^{-1}) (BG11 without carbon source and 45 ppm $NaHCO_3$) whereas lower than with light intensity of 2500 to 3500 lux.[8,26] 10mM concentration of urea has resulted in a similar 5 mM concentration of nitrates.[3] In the present study, urea has resulted in enhanced biomass growth at enhanced light intensities.

Chlorella sp was only successful in open pond cultivation whereas the *Nannochloropsis* and *Picochlorum* were not successful for the fluctuating light conditions.[14] However, *Chlorella* was able to grow under maximum sunlit conditions. But 400 mM salinity has increased stress and the production of stress promoters in the *Scenedesmus sp* was reported by Pancha et al.[24] At 240 µmol/m^2/s light intensity, the photosynthetic efficiency resulted to be high at different L/D cycle frequencies.[19] Similar enhanced photosynthetic efficiency was observed at laboratory conditions rather than room and sunlight conditions. The reason is the very low diffused light in the room which is insufficient to activate the PSII. Under sunlight conditions, due to the higher amount of light intensity, the number of cells are comparatively less to absorb the light falling on that area.

Under sunlight conditions, *Scenedesmus* spare was found to settle at the bottom of the reactor. This may either be due to exposure to high light intensity or due to the excretion of secondary metabolites to overcome the stress of high light intensity. On comparing the photosynthetic efficiency under different light conditions, it is evident that photosynthetic efficiency was low at a low light intensity and initially increases with an increase in light intensity. But at the very high light intensity, the efficiency was found to decrease.[27] This is because the light intensity available is more than that of need. Hence flashing light is employed to reduce the photo-inhibition.[2,15] Excessive light absorption with limited nitrogen resulted in the TAG accumulation.[17] Similar results were observed in this current work under sunlight conditions.

Conclusion

Indigenous microalgae, *Scenedesmus obtusiusculus* cultivated in bubble-column photo-bioreactor resulted in maximum CO_2 fixation rate and enhanced lipid accumulation in nitrogen starvation conditions[30]. On comparing the microwave and sonication, sonication resulted in a higher lipid yield.[11] However, in our current research, osmotic shock yields a better result than the other methods. This may be due to the variation in the cell wall properties of the species.

Although there is a difference in photosynthetic efficiency from laboratory conditions, the species can sustain the fluctuations in light intensity under sunlight conditions with a considerable increase in biomass yield. The higher biomass productivity of *Scenedesmus arcuatus almeriensis* is achieved under outdoor conditions of light intensity 650 to 1625 µE m^{-2} s^{-1}. This is in agreement with our results. Hence there is a high scope for this species to be used on a large scale under sunlight conditions for applications like CO_2 sequestration (since carbon uptake is high), biodiesel production and for production of other valuable products.

Acknowledgement

We sincerely thank the Department of Science and Technology (DST) for giving us an opportunity to work on microalgae for CO_2 mitigation (DST/IS-STAC/ CO2-SR-12/07).

References

1. Aboudheir A., Tontiwachwuthikul P., Chakma A. and Idem R., Kinetics of the reactive absorption of carbon dioxide in high CO2-loaded, concentrated aqueous monoethanolamine solutions, *Chem Eng Sci*., https://doi.org/10.1016/j.ces.2003.08.014, **58**, 5195–5210 **(2003)**

2. Abu-Ghosh S., Fixler D., Dubinsky Z. and Iluz D., Flashing light in microalgae biotechnology, *Bioresour. Technol.*, **203**, 357–363 **(2016)**

3. Arumugam M., Agarwal A., Arya M.C. and Ahmed Z., Influence of nitrogen sources on biomass productivity of microalgae Scenedesmus bijugatus, *Bioresour Technol*, https://doi.org/10.1016/j.biortech.2012.12.159, **131**, 246–249 **(2013)**

4. Bouterfas R., Belkoura M. and Dauta A., The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake, *Limnetica*, **25**, 647–656 **(2006)**

5. Chandra R., Goswami D. and Biotech E., <Scenedesmus dimorphus and Scenedesmus quadricauda two potent indigenous microalgae strains for biomass production and CO2 mitigation.pdf>, *J Algal Biomass Utln*, **2**, 42–49 **(2011)**

6. Converti A. et al, Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production, *Chem Eng Process Process Intensif*, https://doi.org/10.1016/j.cep.2009.03.006, **48**, 1146–1151 **(2009)**

7. Darvehei P., Bahri P.A. and Moheimani N.R., Model development for the growth of microalgae: A review, *Renew. Sustain. Energy Rev.*, **97**, 233–258 **(2018)**

8. Devgoswami C.R. et al, Studies on the growth behavior of chlorella, haematococcus and scenedesmus sp. in culture media with different concentrations of sodium bicarbonate and carbon dioxide gas, *African J Biotechnol*., https://doi.org/10.5897/AJB11.888 **(2011)**

9. Gomes V.G. and Yee K.W.K., Pressure swing adsorption for carbon dioxide sequestration from exhaust gases, *Sep Purif Technol*, https://doi.org/10.1016/S1383-5866(02)00064-3, **28,** 161–171 **(2002)**

10. Gong Q. et al, Effects of Light and pH on Cell Density of Chlorella Vulgaris, *Energy Procedia*, https://doi.org/10.1016/j.egypro.2014.12.064, **61**, 2012–2015 **(2014)**

11. Guldhe A., Singh B., Rawat I. and Bux F., Synthesis of biodiesel from Scenedesmus sp. by microwave and ultrasound assisted in situ transesterification using tungstated zirconia as a solid acid catalyst, *Chem Eng Res Des*, https://doi.org/10.1016/j.cherd.2014.05.012, **92**, 1503–1511 **(2014)**

12. Hadiyanto H., Sumarno S., Nur Rostika R. and Abyor Handayani N., Biofixation of Carbon dioxide by Chlamydomonas sp. in a Tubular Photobioreactor, *Int J Renew Energy Dev*, https://doi.org/10.14710/ijred.1.1.10-14, **1**, 10–14 **(2012)**

13. Hanagata N. et al, Tolerance of microalgae to high CO2 and high temperature, *Phytochemistry*, https://doi.org/10.1016/0031-9422(92)83682-O, **31**, 3345–3348 **(1992)**

14. Huesemann M. et al, A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures, *Algal Res*, https://doi.org/10.1016/j.algal.2015.11.008, **13,** 195–206 **(2016)**

15. Iasimone F. et al, Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics, *J Environ Manage*, https://doi.org/10.1016/j.jenvman.2018.07.024, **223,** 1078–1085 **(2018)**

16. Jeong M.L., Gillis J.M. and Hwang J.Y., Carbon Dioxide Mitigation by Microalgal Photosynthesis, *Bull Korean Chem Soc*, https://doi.org/10.5012/bkcs.2003.24.12.1763, **24**, 1763–1766 **(2003)**

17. Klok A.J., Martens D.E., Wijffels R.H. and Lamers P.P., Simultaneous growth and neutral lipid accumulation in microalgae, *Bioresour Technol*, https://doi.org/10.1016/j.biortech.2013.02.006, **134**, 233–243 **(2013)**

18. Lee S., Song H.J., Maken S. and Park J.W., Kinetics of CO2 absorption in aqueous sodium glycinate solutions, *Ind Eng Chem Res*, https://doi.org/10.1021/ie061270e, **46**, 1578–1583 **(2007)**

19. Liao Q., Li L., Chen R. and Zhu X., A novel photobioreactor generating the light/dark cycle to improve microalgae cultivation, *Bioresour Technol*, https://doi.org/10.1021/ie061270e**, 161**, 186–191 **(2014)**

20. Martínez Sancho M., Jiménez Castillo J. and El Yousfi F., Photoautotrophic consumption of phosphorus by Scenedesmus obliquus in a continuous culture. Influence of light intensity, *Process Biochem*, https://doi.org/10.1016/S0032-9592(99)00006-0, **34**, 811–818 **(1999)**

21. Mutale-joan C. et al, Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of Solanum lycopersicum L., *Sci Rep*, https://doi.org/10.1038/s41598-020-59840-4, **10**,1–12 **(2020)**

22. Nithiya E.M., Fenila F., Vasumathi K.K. and Premalatha M., Cultivation of *scenedesmus* sp. using optimized minimal nutrients and flocculants – a potential platform for mass cultivation, *Environmental Technology*, **3**,1-14 **(2018)**

23. Pancha I. et al, Bicarbonate supplementation enhanced biofuel production potential as well as nutritional stress mitigation in the microalgae Scenedesmus sp. CCNM 1077, *Bioresour Technol*, https://doi.org/10.1016/j.biortech.2015.06.107, **193**, 315–323 **(2015a)**

24. Pancha I. et al, Salinity induced oxidative stress enhanced biofuel production potential of microalgae Scenedesmus sp. CCNM 1077, *Bioresour Technol*, https://doi.org/10.1016/j.biortech.2015.04.017, **189,** 341–348 **(2015b)**

25. Rinker E.B., Ashour S.S. and Sandall O.C., Absorption of carbon dioxide into aqueous blends of diethanolamine and methyldiethanolamine, *Ind Eng Chem Res*, https://doi.org/10.1021/ie990850r, **39,** 4346–4356 **(2000)**

26. Sánchez J.F. et al, Biomass and lutein productivity of Scenedesmus almeriensis: Influence of irradiance, dilution rate and temperature, *Appl Microbiol Biotechnol*, https://doi.org/10.1007/s00253-008-1494-2, **79**, 719–729 **(2008)**

27. Singh S.P. and Singh P., Effect of temperature and light on the growth of algae species: A review, *Renew. Sustain. Energy Rev.*, **50**, 431–444 **(2015)**

28. Solovchenko A.E. et al, Effects of light intensity and nitrogen

starvation on growth, total fatty acids and arachidonic acid in the green microalga Parietochloris incisa, *J Appl Phycol*, https://doi.org/10.1007/s10811-007-9233-0, **20**, 245–251 **(2008)**

29. Stanbury P.F., Whitaker A. and Hall S.J., Principles of Fermentation Technology: Third Edition **(2016)**

30. Toledo-Cervantes A., Morales M., Novelo E. and Revah S., Carbon dioxide fixation and lipid storage by Scenedesmus obtusiusculus, *Bioresour Technol*, https://doi.org/10.1016/ j.biortech.2012.12.081, **130,** 652–658 **(2013)**

31. Vasumathi K.K., Premalatha M. and Subramanian P., Parameters influencing the design of photobioreactor for the growth of microalgae, *Renew Sustain Energy Rev*, https://doi.org/10.1016/j.rser.2012.06.013, **16**, 5443–5450 **(2012)**

32. Vasumathi K.K., Premalatha M. and Subramanian P., Experimental studies on the effect of harvesting interval on yield of Scenedesmus arcuatus var. capitatus, *Ecol Eng*, https://doi.org/10.1016/j.ecoleng.2013.06.012, **58**, 13–16 **(2013)**

33. Wilson E.J., Johnson T.L. and Keith D.W., Regulating the ultimate sink: Managing the risks of geologic CO2 storage, *Environ. Sci. Technol.*, **37**, 3476–3483 **(2003)**

34. Xin L., Hong-ying H. and Yu-ping Z., Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature, *Bioresour Technol*, https://doi.org/10.1016/j.biortech.2010.10.055, **102**, 3098–3102 **(2011)**.

(Received 05th January 2021, accepted 10th March 2021)