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CARBON DIOXIDE FIXATION BY *CHLORELLA MINUTISSIMA* BATCH CULTURES IN A STIRRED TANK BIOREACTOR

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ABSTRACT

Increasing concentrations of CO_2 in the atmosphere is causing severe environmental destruction to the earth. To capture the excess CO_2, its microalgal sequestration is increasingly being explored. This technique could become a profitable industry that would help mitigate global warming and produce a large number of value-added products like pigments, carotenoids, carbohydrates, lipids, etc. In this regard, three microalgal strains viz., Calothrix sp., Spirulina platensis and Chlorella minutissima were studied for their growth characteristics. Based upon the kinetic parameters, possible by-products, tolerance to CO_2, etc., the best strain was selected for further studies. The optimum pH, photoperiod, nitrate concentration and light intensity for this strain were experimentally determined. Also, studies were conducted with and without baffles, with varying aeration rates and with two different impellers and i.e., marine propeller and disc turbine impeller, in the presence of air alone and a mixture containing air with 15% CO_2. Among the three strains studied, C. minutissima was found to be the best strain for further work. It has maximum biomass productivity at pH 6, photoperiod of 14 h light:10 h dark cycles, 5 g/l nitrate and 6000 lx light intensity. Runs with baffles yielded higher biomass. Marine propeller gave better biomass yields in both runs i.e., with and without additional CO_2. Also, the optimum aeration rate was determined to be 1 lpm. The carbohydrate, lipid, protein, chlorophyll and carotenoid content of the biomass were estimated.

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Introduction

Global warming due to increasing concentrations of greenhouse gases like CO_2, from anthropogenic sources is becoming a worldwide concern. This phenomenon may cause severe malignant environmental problems like increase in the average surface temperature of the planet, which may lead to alteration in precipitation pattern, rise in level of oceans, accelerated glacial melting, etc. (7, 9, 15, 48, 60). In recent years, many studies have been carried out to investigate the various strategies for CO_2 capture and sequestration. Many chemical and physical methods like absorption, adsorption, and membrane separation, injection into oceans or geologic seams have been examined to separate CO_2 from flue gas or to permanently store it. But these techniques for capturing CO_2 are not feasible due to their high cost or use of so much energy, so that the mitigation benefits become marginal. Hence, the need for an alternate sequestration technology arises (3, 25, 50, 52, 54).

Microalgal biofixation of CO_2 is a promising way to completely sequester CO_2 by converting it into biomass by way of photosynthesis (39, 48). CO_2 mitigation using microalgal systems has several technological advantages like mild conditions for fixation and non-requirement for further disposal of trapped CO_2. The carbon fixed by microalgae is incorporated into carbohydrates and lipids. So, energy, chemicals or foods can be produced from algal biomass (48, 55, 60). Also, as microalgae grow in aqueous environments, directly passing the flue gases through this medium is a cost effective and efficient means of capturing CO_2 (40). Algal cultures have higher rates of CO_2 fixation than land plants. They are also better suited for incorporation into industrial processes than other photosynthetic systems using higher plants. Many microalgal systems are able to grow in highly saline and alkaline conditions, tolerate fluctuating temperatures, high CO_2 concentrations and varying light intensities (5, 34).

Various sequestration studies have been carried out using different algal strains based upon their tolerance to higher concentrations of CO_2 and other flue gas components, temperature, pH stabilities and the various value added products possible from the biomass (13, 27, 54, 55, 60). *Spirulina*, *Chlorella*, *Aphanothece* and *Scenedesmus* species have been the most targeted microalgae (8, 12, 14, 18, 30, 32, 35, 38, 46, 58). *Spirulina* sp. has been closely investigated for the production of nutraceutical compounds like antioxidants, vitamins, industrially important materials like PHB and hydrogen gas (1, 10, 23, 41). It has been reported to tolerate up to 18% CO_2 (12). *Chlorella* sp., on the other hand, has been found to tolerate up to 50% CO_2 (54, 55, 60). Certain *Chlorella* strains are also able to grow well in presence of low concentrations of SO_2 and NO_x, indicating that they can be used for direct CO_2 fixation from actual flue gas (27).

Most algal cultures are carried out in photobioreactors. They allow better control of cultivation conditions than open

systems like ponds. Higher biomass productivity, prevention of contamination and non-requirement of large areas of land are other factors favouring the use of photobioreactors (36, 56). Various types of reactor configurations have been studied for the culture of microalgae like tubular, LED based, flat plate, vertical column photobioreactors, etc. (26, 33, 56). Modelling of these photobioreactors for cultivation of algal systems has been attempted (2, 4). Very few investigations have been carried out in stirred tank photobioreactors. They have several advantages over other reactor configurations. These can be scaled up easily. They have a very effective stirring mechanism. Hence, mass transfer rates and light dispersion are very high. This leads to a lower incidence of dark zones inside the reactor and higher biomass productivity (29, 37, 56). So, in the present study, stirred tank photobioreactor was chosen for culturing microalgae.

The objective of the present study was to perform a comparative study of growth of three algal strains. Based upon their doubling time, maximum specific growth rate, by-products possible, easiness of quantification of biomass, etc., a potential strain was selected. This strain was taken up for further studies. The pH, photoperiod, initial nitrate concentration and light intensity for this strain were optimised. This strain was then cultured in a stirred tank photobioreactor and effect of baffles, various impeller types, and aeration rates on biomass productivity was studied. Also, the various value added products from biomass like lipids, protein, chlorophyll, total carotenoids, beta carotenoids, etc. were estimated.

Materials and Methods

Microalgal cultures, medium and chemicals

The three algal strains identified for the present study were the cyanobacteria *Spirulina platensis* and *Calothrix* sp. and the green alga *Chlorella minutissima*. The medium used for the maintenance of inoculum and cultivation of *Spirulina platensis* was Zarrouk's medium containing (g/l): $NaHCO_3$, 16.8 g; K_2HPO_4, 0.5 g; $NaNO_3$, 2.5 g; NaCl, 1.0 g; $MgSO_4.7H_2O$, 0.2 g; $FeSO_4.7H_2O$, 0.01 g; K_2SO_4, 1.0 g; $CaCl_2.2H_2O$, 0.04 g; and micronutrients (11). pH was maintained at 9.5. *Calothrix* sp. was maintained in Bristol's modified medium which consists (per litre): $NaNO_3$, 0.75 g; KH_2PO_4, 0.175 g; K_2HPO_4, 0.075 g; $MgSO_4.7H_2O$, 0.075 g; $CaCl_2.2H_2O$, 0.025 g; NaCl, 0.025 g; $FeCl_3$, 5 mg; $ZnSO_4.7H_2O$, 0.287 mg; $MnSO_4.H_2O$, 0.169 mg; H_3BO_3, 0.061 mg; $CuSO_4.5H_2O$, 0.0025 mg and $(NH_4)_6Mo_7O_{24}.7H_2O$, 0.00124 mg (19). M4N medium was used for the culture of *C. minutissima* which contained (per litre): KNO_3, 5 g; $MgSO_4.7H_2O$, 2.5 g; KH_2PO_4, 1.25 g; $FeSO_4.7H_2O$, 3 mg; H_3BO_3, 2.86 mg; $MnSO_4.7H_2O$, 2.5 mg; $ZnSO_4.7H_2O$, 0.222 mg; $CuSO_4.5H_2O$, 0.079 mg and Na_2MoO_4, 0.021 mg (58). *Calothrix* cultures were maintained at pH 7.2 and *C. minutissima* at pH 6.0.

Growth curve studies in shake flask

The three microalgae were cultivated in 250 ml Erlenmeyer flasks with 100 ml working volume. Cultures were maintained at 27ºC and 110 rpm. Illumination was provided by white fluorescent light of 20 W. The luminous intensity was 3500 lx. The cultures were illuminated in 14 h light/10 h dark photoperiods (13, 58, 60). The cultures were incubated till the stationary phase of the growth cycle was reached. Samples were collected at regular intervals and the dry weight of the biomass estimated. All the experiments were carried out in triplicates. Based upon the lowest doubling time, maximum specific growth rate, maximum productivity, value added products possible from the biomass and higher tolerance to CO_2 from literature, the best strain was selected for further studies.

Optimisation of initial pH

In a photobioreactor, pH of the medium is an important factor that significantly affects the growth of the algae (29). Hence, to determine the effect of initial medium pH on the growth of the algae, the pH was varied in the range 4-8. The pH was adjusted using 0.1N NaOH or 0.1N HCl using a pH meter (Digital pH meter 335, Systronics, Ahmedabad, India). Samples were aseptically collected at 24 h intervals and the dry weight of the biomass was estimated.

Optimisation of photoperiod

Duration of photoperiod is an influential factor in the production of biomass (32). To study the effect of various photoperiods on biomass productivity of *Chlorella minutissima*, various cultures were carried out with different photoperiods. The various photoperiods chosen for the study were 6:18, 12:12, 14:10 and 18:6 (light:dark) cycles.

Optimisation of light intensity

Light intensity plays a significant role in the culture of photosynthetic organisms. Hence the effect of this parameter on the biomass yield of *C. minutissima* was studied by growing the strain under three different light intensities viz., 2000, 6000 and 10000 lx.

Optimisation of medium nitrate concentration

Nitrate and phosphate are the major nutrients for a non nitrogen fixing microalgae. The N:P ratio in the media significantly affects the cell density and biomass yield. Experiments were conducted to determine the optimum nitrate concentration for the strain. The alga was grown in media containing 0, 2.5, 5.0, 7.5 and 10.0 g/l nitrate.

Effect of impeller type

The type of impeller has a profound effect on the hydrodynamics of the reactor. This in turn affects the mass transfer of the gases including CO_2, nutrients, mixing time and light conversion in the photobioreactor (44, 48, 56). So, algal culture was carried out with two different impellers - disc turbine and marine propeller, to determine the optimal conditions to improve mass transfer within the reactor. The cultivation was carried out in a 2 litre stirred tank bioreactor (Bioflo 110 fermentor, New Brunswick Scientific, New Jersey, USA) with a working volume of 1.8 litre. Aeration rate was maintained at 0.56 vvm.

TABLE 1

Kinetic parameters of the algal strains

Parameters	*Calothrix* sp.	*Spirulina platensis*	*Chlorella minutissima*
μ_{max}, (h^{-1})	0.00943	0.0098	0.0105
P_{max} (g/l/h)	0.00074	0.00288	0.0032
t_d (h)	73.5	70.73	66.01
Duration of lag phase (h)	60	48	30
Exponential phase growth period	60^{th}-168^{th} h	48^{th}-288^{th} h	30^{th}-234^{th} h

The light intensity was 6000 lx and photoperiod was 14 h light/10 h dark cycles. The initial pH of the culture medium was 6.0 and was left uncontrolled throughout the culture.

Effect of baffles

Baffles play a major role in the mixing efficiency in a reactor. To determine the effect of baffles in algal cultivation in bioreactor, experiments were carried out with and without baffles. The baffles used here were 4 in number.

Effect of aeration rates

To investigate the effect of aeration rates on the biomass yield of *C. minutissima*, air was supplied to the reactor at varying aeration rates of 0.5, 1, 1.5 and 2.0 lpm.

Carbon dioxide fixation studies

To determine the potential of using of the selected algal strain for carbon dioxide sequestration, the strains were cultured with 15% CO_2. Runs were carried out with both impeller types. The inoculum used was pre-adapted to CO_2.

Estimation of dry weight of biomass

Samples were collected aseptically. For *S. platensis* and *C. minutissima*, the absorbance of the samples at 670 and 660 nm, respectively, were measured using a visible spectrophotometer (Spectrochem NV201, Aimil Ltd., India). The optical density was converted to dry weight using a calibration curve (12, 27, 55). Quantification of biomass by absorbance measurement was not possible for *Calothrix* sp. Hence, the samples were filtered using Whatman's No. 1 filter paper and dried at 60ºC overnight to obtain the dry weight of the biomass (43).

Kinetic parameters

The biomass values were plotted against time to construct the growth curves. From the exponential phase, the specific growth rate (μ, d^{-1}/h^{-1}) was calculated according to the equation $\mu=(\ln X_2-\ln X_1)/(t_2-t_1)$, where, X_2 and X_1 are the dry biomass weight (g/l) at time t_2 and t_1, respectively. From the different μ values, the maximum specific growth rate (μ_{max}, d^{-1} or h^{-1}) was determined. The cell doubling time was estimated as t_d (d or h)=$\ln 2/\mu_{max}$. The maximum biomass obtained was designated as X_{max} (g/l). Productivity was calculated using the equation, $P=(X_t-X_o)/(t_t-t_o)$, where X_t is the biomass concentration at time t and X_o, the initial biomass concentration at inoculation time, t_o. P_{max} (g/l/d or g/l/h) was designated as the maximum productivity (12, 14, 19).

Estimation of value-added products

The total carbohydrate content, lipid content and protein content of the biomass was estimated. The protocols used were phenol sulphuric acid method, Bligh and dyer's method and Lowry's method, respectively. Chlorophyll and total carotenoid were estimated by extraction with chloroform methanol mixture using Wellburn's equation (57) Beta carotenoids were estimated by extraction with methanol by the protocol of Nagata and Yashimata, 1992 (35).

Results and Discussion

Growth, morphological characteristics and selection of a potential strain

Fig. 1 shows the growth pattern of the three algal cells. Growth was measured in terms of biomass dry weight (g/l). Biomass can be more easily measured by using absorbance than by measuring cell dry weight directly. So, the relationship between optical density and cell dry weight for *C. minutissima* and *S. platensis* were established by linear regression (9). It was found that optical density precisely predicted the dry weight (R^2=0.998). Hence, OD values were used to calculate the biomass of these two strains. **Table 1** shows the μ_{max}, P_{max}, t_d, duration of lag phase and exponential phase growth period for the three strains.

It has been shown by other investigations that the X_{max} values were in the range of 0.82-0.92 g/l after 600 hrs of cultivation for *S. platensis* (10). But, in the present work, an X_{max} value of 1.02 g/l was obtained after 288 hrs of culture. Certain strains of *S. platensis* like LEB - 52 have been found to have a μ_{max} of 0.073/day and a P_{max} of 0.0416 g/l/d (45). The strain in the present study has a higher μ_{max} and P_{max}. μ_{max} and P_{max} for *Chlorella kessleri* were determined to be 0.257/d and 0.090 g/l/d. Maximum biomass attained was 1.45 g/l after 20 days of cultivation (13). These values were found to be similar to the *Chlorella* strain in the present study. Much literature is not available for studies on *Calothrix* sp.

Numerous studies have been carried out for determining the kinetic parameters of other potential microalgal strains. *Galdieria sulphuraria*, which is a prospective producer of phycocyanin has been reported to have a μ_{max} of 0.10/d (51). *Chlamydomonas reinhardtii*, an established biofuel producer, has a doubling time as low as 5 h under laboratory conditions and 24 h under ambient culture conditions (47). μ_{max} was

estimated at 0.232/d for *Haematococcus pluvialis*, which has high application potential in industrial production of astaxanthin and various other nutraceuticals (24).

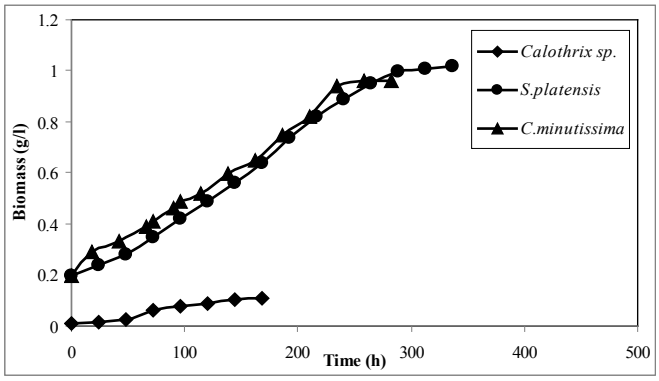
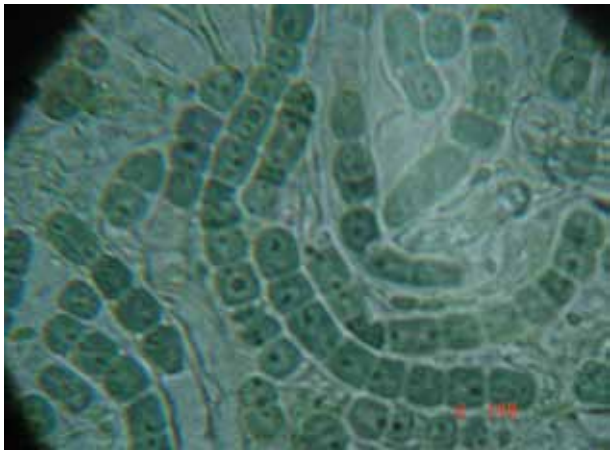


Fig. 1. Growth curves of the three algal strains

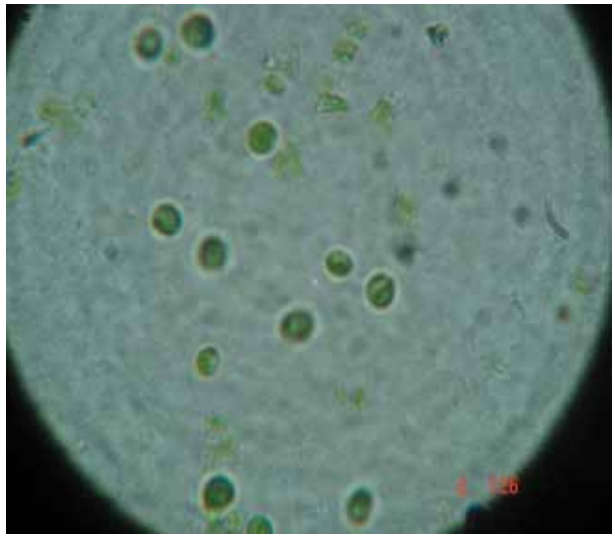
Based up on the experimental results, the μ_{max}, P_{max}, t_d and X_{max} of all the strains were compared. Since μ_{max}, P_{max} and X_{max} of *Calothrix* sp. were very low compared to the other strains, and t_d was higher, this strain was rejected for further studies. Even though many by-products like chlorophyll, lipids, proteins, etc. are possible from *Calothrix* sp., the estimation of biomass is very difficult (43). The biomass tends to form a sheath and stick to the walls of the flask. On comparison of μ_{max}, P_{max}, t_d, number of days for cultivation, tolerance to CO_2, etc., *C. minutissima* was found to be superior strain for further studies, even though *Spirulina* and *Chlorella* strains have similar maximum biomass yield and high number of by-products.

Fig. 2 shows the morphology of the three strains under the conditions used in this study. *Calothrix* is a filamentous, heterocystous cyanobacteria. These form a gelatinous colony containing a number of filaments. Hence, obtaining a single cell is very difficult. On the other hand, individual cells of the non-heterocystosus, filamentous cyanobacteria, *S. platensis* can be easily viewed. These cells multiply by binary fission and show easily visible transverse cross walls. *C. minutissima* is a unicellular eukaryotic alga and its nucleus is clearly visible in **Fig. 2**. It is spherical in shape and highly motile.



a)

b)



c)

Fig. 2. Microscopic view of the algal strains under 100X magnification (a) *Calothrix Sp.*; (b) *Spirulina platensis*; (c) *Chlorella minutissima*

Optimisation of initial pH

Medium pH is an important factor which significantly affects the growth of the algae. The variation in pH affects the solubility and availability of nutrients, enzyme activity, and transport of substrates across plasma membrane and electron transport in respiration and photosynthesis (13). So, pH was optimised for the microalga *C. minutissima*. The growth patterns under various pH regimes, varying from 4 to 8 are given in **Fig. 3**.

As is evident from the figure, the best growth was observed at pH 6, where the μ_{max} is 0.226/d and X_{max} 1.479 g/l. The organism was found to grow well between pH 5 and pH 7. So, a pH shift above 5 was found not to negatively affect the growth. But, when the pH was lower than 5 or higher than 7, the growth was noticeably reduced. When *C. minutissima* was cultured at pH 4, the growth ceased after 9 days, whereas at pH 8, growth continued until 11 days. The growth pattern was similar in the pH range 5-7. The pH shift in media, which was due to non-controlling of pH, did not seem to affect the growth.

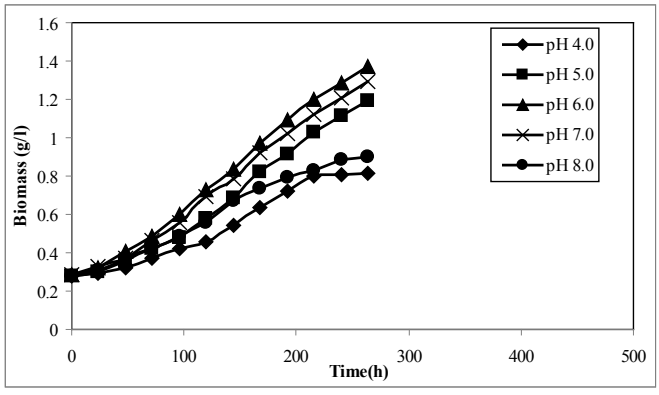


Fig. 3. Growth pattern of *C. minutissima* at varying pH

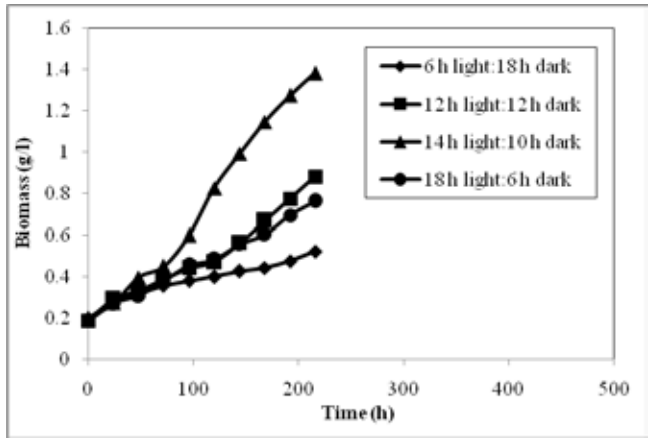


Fig. 4. Growth curves of *C. minutissima* in various light cycles

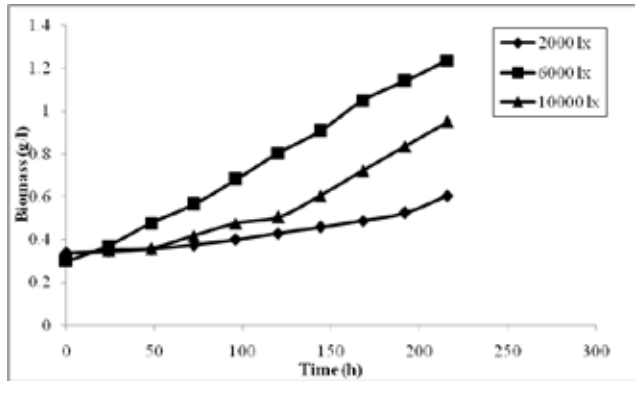


Fig. 5. Growth curve of *C. minutissima* under varying light intensities

Optimisation of photoperiod

The light regimes under which a microalgae is cultivated is an important factor in the productivity and yield of the algae. A detailed study of the effect of the various light regimes is important when the microalgae are industrially cultivated. Researchers have optimised the photoperiod for *Aphanothece microscopica Nageli*. They found that maximum productivity was obtained under constant light supply conditions (32). But in the present study as is shown in **Fig. 4**, it was found that a maximum biomass yield of 1.4903 g/l was obtained at the end of 9 days of cultivation for cultures grown under 14 hours of lighting and 10 hours of darkness. Least productivity was observed for cultures grown under 18 hours of darkness. In this case the X_{max} was only 0.82 g/l. Several investigations have reported that light is a major limiting substrate in culture of algal systems. Aeration rates, cell concentration inside the culture system, all affect the light availability inside the photobioreactors and in turn, it affects the productivity of the algal systems (20, 22, 21).

Optimisation of light intensity

From the results obtained, it was found that light intensity has significant impact on biomass productivity of *C. minutissima* cells. At low light intensity of 2000 lx, the biomass did not increase even two fold after 9 days of cultivation as it is evident from **Fig. 5**. A high X_{max} of 1.2366 g/l was obtained when the cells were grown under light intensity of 6000 lx. At 10000 lx also the strain could grow well, but the productivity was lower. Investigations have revealed that the photosynthetic efficiency could decrease due to high or low light treatment. High light intensity can lead to photobleaching of photosynthetic pigments, peroxidation of membrane lipids and degradation of DNA. These effects result from the production of reactive oxygen species, malondialdehyde and photosystem II damage (6, 16, 17, 42). Low light intensities have been reported to increase chlorophyll and carotenoid content and decrease growth rate and cell density (28).

Optimisation of medium nitrate concentration

As indicated at **Fig. 6** the optimum nitrate concentration for *C. minutissima* growth was 5 g/l. The μ_{max} in this case was found to be 0.1875/day. The maximum biomass obtained at the end of 9 days of cultivation was 1.2366 g/l and the P_{max} under this condition was found to be 0.10723 g/l/d during 7th day of growth. Growth was found to be almost similar when the cells were grown with 2.5 or 7.5 g/l potassium nitrate. In these cases, the X_{max} values obtained were 1.0508 and 0.9779 g/l, respectively. But, when the nitrate concentration was 10.0 g/l, a substrate inhibition was observed. The maximum biomass yield was reduced to 0.8769 g/l. It was observed that when *C. minutissima* cells were grown in media without nitrate, the cells started decolourising after 3-4 days. The biomass also did not increase considerably and the yield was as low as 0.37422 g/l at the end of 9 days of cultivation. Also, in runs without nitrate or very low amounts of nitrate, the cells were found to stick to the walls of the shake flask. Investigations have revealed that nitrogen starvation/limitation leads to reduction of major and accessory photosynthetic pigments, impairment of photosynthesis due to loss of one major Rubisco isoenzyme, reduced synthesis of lipids and fatty acids, reduced nitrate reductase activities and enhanced production of hydrogen peroxide (49).

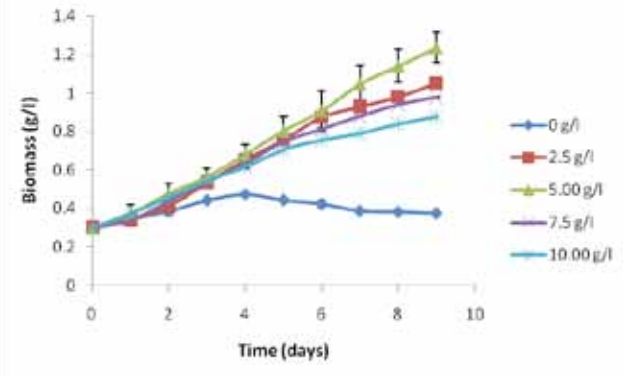


Fig. 6. Growth curve of *C. minutissima* under varying nitrate concentrations

Effect of impeller type

From the present study, it can be invariably concluded that the type of impeller has a profound effect on the performance of the reactor. From the experimental results, for culture with air only (**Fig. 7**), it was found that *C. minutissima* when cultured with disc turbine impeller had a μ_{max} of 0.0361/h and X_{max} of 1.053 g/l. On the other hand, with marine propeller, μ_{max} was 0.0409/h and maximum biomass was 1.27 g/l after 240 hours of cultivation. For both runs, lag phase lasted only for about 25 hours.

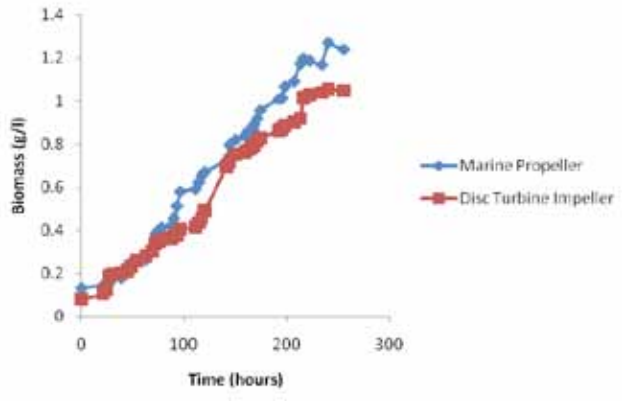


Fig. 7. Growth pattern of *C. minutissima* with varying impellers

From these findings, it might be concluded that marine propeller is superior for algal culture. The reason for this is the different flow pattern due to these impellers. Disc turbines are better suited when the air flow rates are very high. They can break up a fast air stream without themselves becoming flooded in air bubbles. They have a radial flow pattern. When the air flow rate is lower, marine propeller is the preferred choice. The air bubbles travelling up from the sparger do not initially hit any surface before dispersion by the blades, unlike the disc turbine (53). Hence, these differences in flow patterns were observed to have a great impact on the algal culture in bioreactor. In the present study, since the flow rate was very low, about 0.9 lpm, marine propeller was found to be the better suited impeller.

Effect of baffles

Baffles are significant in bioreactor cultivation, particularly when stirred tank reactors are used. They increase the residence time of the gas bubbles and improves the mixing efficiency without any addition of energy. Various types of baffles have been studied in this regard (48). In the present investigation, it was found that culture of algal cells with baffles yielded more biomass (**Fig. 8**). Also, when baffles were not used, the cells had a tendency to stick to the walls of the reactor. The X_{max} values when *C. minutissima* was grown in reactor with and without baffles were 1.10052 and 0.796 g/l respectively.

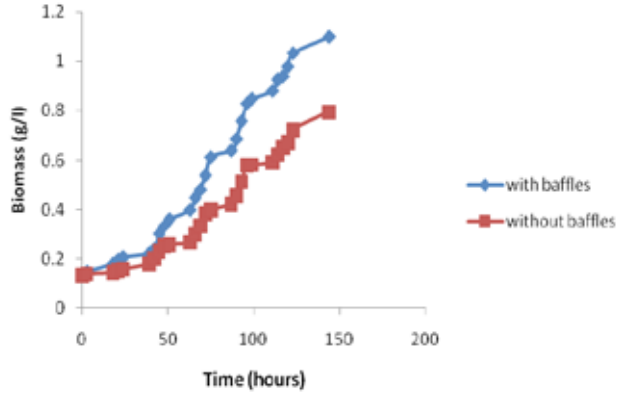


Fig. 8. Growth curve of *C. minutissima* with and without baffles

Effect of aeration rates

C. minutissima are small eukaryotic organisms and are susceptible to high shear rates. High aeration rates were found to be detrimental to the growth of algae. At 2 lpm, the biomass yield was only 0.56785 g/l at the end of 120 h cultivation (**Fig. 9**). On the other hand, culture at very low flow rate of 0.5 lpm resulted in even lower productivities. In this case, the cells were observed to sediment to the bottom of the reactor. The X_{max} was as low as 0.4123 g/l. The best productivity was obtained with 1 lpm flow rate. The X_{max} in this case was 0.97794 g/l and was considerably higher than the other obtained biomass yields.

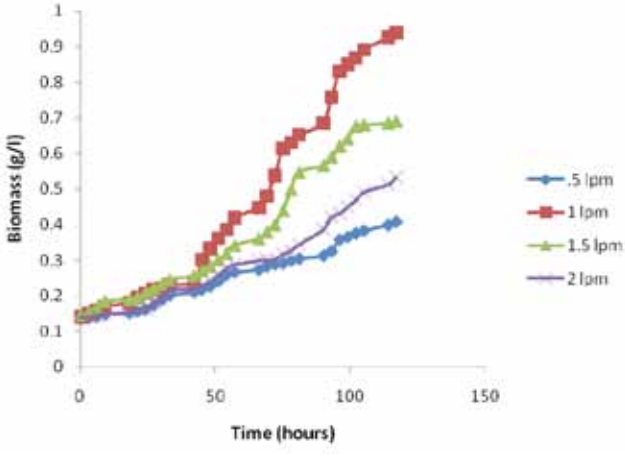


Fig. 9. Growth curve of *C. minutissima* under varying aeration rates

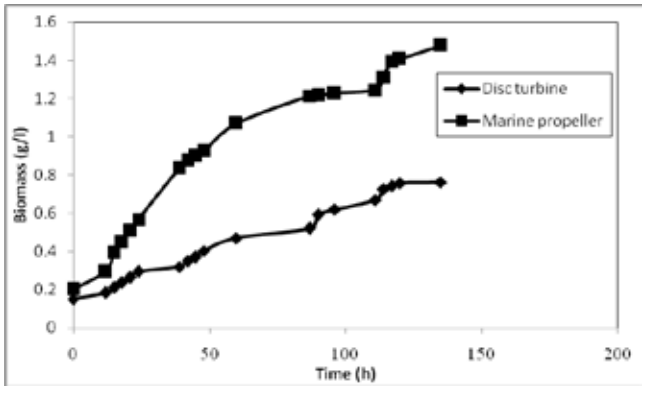


Fig. 10. Growth pattern of *C. minutissima* with varying impellers in presence of 15% CO_2

Carbon dioxide fixation studies

Carbon dioxide sequestration studies were carried out for *C. minutissima* with the two impellers. It was found that for the runs with 15% CO_2, higher biomass values were obtained for trials with marine propeller. X_{max} and μ_{max} values were 0.8824 g/l and 0.0131/h after 138 hours of cultivation for runs with disc turbine propeller. The corresponding values with marine propeller were 1.596 g/l and 0.047/h after 135 hours of culture, as it is evident from **Fig. 10**. The X_{max} values were much higher when cells were cultured with CO_2, in both cases. Thus, it can be inferred that *C. minutissima* can be used for CO_2 capture from flue gas.

The duration of lag phase in both these trials were greatly reduced as the cells were adapted to CO_2 before actual culture in bioreactor. When the morphological characteristics of the *C. minutissima* cells grown with and without CO_2 are compared, a marked difference can be observed as it is evident from **Fig. 11**. The cells appear to be larger in size and much more actively dividing when grown with CO_2.

Many studies have been carried out which have explored the potential of *Chlorella* species for CO_2 sequestration. Several strains which are capable of tolerating up to 50% CO_2 have been isolated and studied (8, 9, 13, 15, 55, 60). Other strains such as *Scenedesmus obliquus*, that have biomass yields comparable to the strain in the present study have also been investigated. They have been shown to endure till 18% CO_2 (12, 14). *Aphanothece microscopic Nageli* has been studied in detail for their CO_2 removal rates in tubular and bubble column photobioreactors (30, 31). *Spirulina platensis*, one of the widely studied microalga has been shown to have a very high biomass yield and culture in presence of CO_2 positively influences its kinetic parameters. They can withstand up to 18% CO_2 (12, 14, 59). With flue gas, the lipid productivity was found to increase 1.9 fold for *Botryococcus braunii*, a microalgae whose lipid content is as high as 21% of biomass (59).

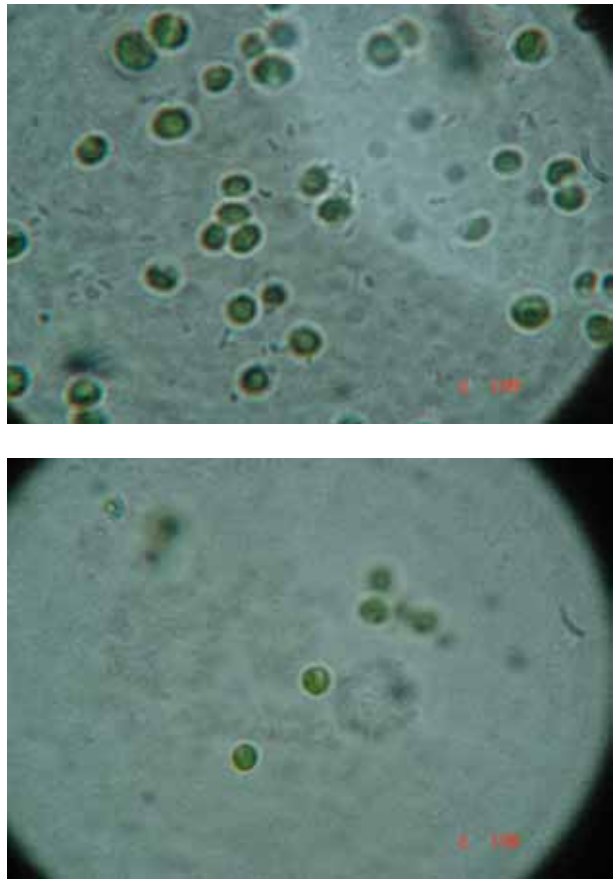
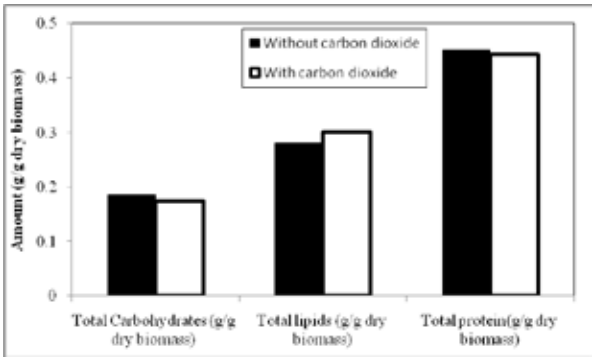


Fig. 11. Microscopic view of algal cells cultured with and without CO_2 under 100X magnification

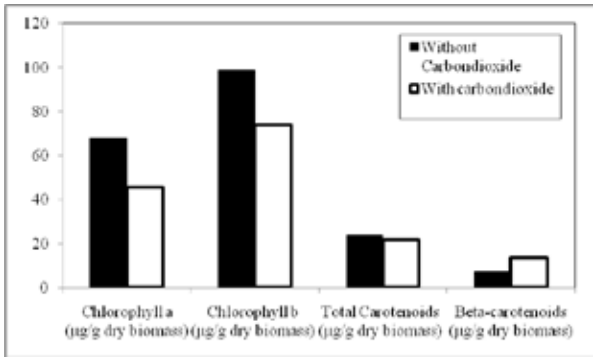
(a) *C. minutissima* cultured with 15% CO_2; (b) *C. minutissima* cultured with air only

Estimation of value-added products

From the results obtained (**Fig. 12**), it was found that *C. minutissima* is a rich source of protein and lipid. The alga has the potential to be used as a single cell protein and for biofuel production. There was not considerable difference between the biomass composition of cells grown with and without CO_2. The carbohydrate content of the cells cultured with and without CO_2 were 0.2 and 0.188 g/g dry biomass, respectively. The lipid content was slightly higher when the cells were cultured with CO_2. The lipid content was 32.56% for cells cultured with CO_2. On the other hand, it was 30.262% for cultivation without CO_2. The protein content was almost similar. The chlorophyll content and total carotenoid content was considerably higher when the cells were cultured without CO_2. Chlorophyll a and b contents were found to be 67.8176 and 98.6767 µg/g dry biomass when alga was cultivated without CO_2. The corresponding values for cells cultured with CO_2 were 45.61142 and 74.0307 µg/g dry biomass. Beta carotenoid was higher when the cells were cultured with CO_2. The results obtained indicate that *C. minutissima* can be used as a source of chlorophyll and carotenoids. Research indicates that *C. minutissima* is the largest producer of chlorophyll pigment.



a)



b)

Fig. 12. Value-added products from biomass

(a) Total carbohydrate, lipid and protein content of biomass and (b) Chlorophyll a and b, total carotenoids and beta carotenoids content of biomass

Conclusions

The results of the selection of potential strain for CO_2 fixation studies, optimisation of pH and studies on effect of impeller types are summarised as follows:

(1) Among the three algal strains studied, *C. minutissima* was selected for further studies due to its lowest t_d, lowest lag phase, highest μ_{max}, highest P_{max} and the possibility of extracting various by-products like pigments, lipids, proteins, etc.

(2) *C. minutissima* was found to grow well in the pH range 5-7, thus implying that it can be cultured in varying environmental conditions. The pH optimum was found to be 6.

(3) The optimum photoperiod for *C. minutissima* was found to be 14:10 light/dark cycles. The alga also grew well in 12:12 dark/light cycles.

(4) Light intensity was found to be a very important factor that determines algal productivity. The best productivity was found to be 6000 lx. Light intensities above or below this value were detrimental to culture.

(5) Nitrate is an important nutrient for non nitrogen fixing strains and is a limiting nutrient in many cases. In the present study, nitrate concentration was optimised and the optimum value was found to be 5.0 g/l, even though it grew well in the concentration range 2.5-7.5 g/l.

(6) In experiments varying impellers, marine propeller emerged as a better impeller in all runs. In runs with both impeller types, the cell biomass was found to be considerably higher when the cells where cultured with CO_2.

(7) Baffles were found to significantly affect the growth rates. Cultures with baffles yielded higher biomass.

(8) The best biomass yield was obtained when the cells were cultured at 1 lpm. At aeration rates above or below this value, the biomass yield was low. At lower aeration rates, the cells were found to settle down. At higher aeration, there was a high turbulence, which might cause the cells to disrupt.

(9) The *C. minutissima* strains were found to be capable of sequestering carbon dioxide. The X_{max} was as high as 1.596 g/l just after 135 hours of cultivation, when *C. minutissima* was cultured with 15% CO_2.

(10) A marked morphological difference was observed when the alga was cultured with CO_2. These cells were larger in size and much more actively dividing than when cultured without CO_2.

(11) The results of estimation of value-added products from biomass indicate that *C. minutissima* can be used as SCP, biofuel, source of chlorophyll and carotenoids.

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