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Improved CO_2 fixation with *Oscillatoria* sp. in response to various supply frequencies of CO_2 supply

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ABSTRACT

Sequestration of CO_2 by microalgae is one of the technically viable options to control the CO_2 emission. CO_2 sequestration by microalgae through photosynthesis also results in large quantity of biomass production with considerable fuel properties. Different types of micro algal species have got CO_2 tolerance to a different level. This work focuses on the tolerance capability of *Oscillatoria* sp. for 100% CO_2, bubbled into the ASN III media. CO_2 supply was given at a flow rate of 300 ml/min for 15 min. 12:12 h of light to dark cycle was followed.CO_2 supply was varied from one to three times a day during the light period. An increasing trend of biomass growth was observed with increased supply frequency of CO_2. An increase of biomass yield of 44.7%, CO_2 fixation of 64.2% and calorific value of 17.6% was observed corresponding to the three times supply frequency of CO_2, compared with the control. Ultimate analysis, stoichiometric analysis and molecular weight of microalgae grown in control and pure CO_2 (100%) gas were compared. Growth of microalgae obtained at various supply frequencies of CO_2 were fitted with logistic model with a correlation coefficient of 0.98. The present studies indicated that the *Oscillatoria* sp. was able to sustain in pure CO_2(100%) gas and may be considered as the species for converting the emissions from the distilleries to energy.

1. Introduction

Increase in CO_2 concentration is the prominent cause of global warming, having massive threats on human life and deleterious effect on environment and climate. [1–4]. CO_2 contributes for 52% of global greenhouse gas emission [5] and it has reached 402.56 ppm in January 2016 with an average rise of every 2 ppm for the past five years [6]. CO_2 levels exceeding beyond 450 ppm in the atmosphere will have adverse effects on the global climate conditions, sea levels and survival of many organisms [7]. However, the CO_2 mitigation techniques such as chemical adsorption, solid adsorbents, membrane technology and cryogenic fractionation have safety and environmental drawbacks in capturing CO_2 from the atmosphere and flue gas [8]. Hence, researches on utilising microalgae for CO_2 sequestration and biofuel generation have gained profound attention among the researchers, policy makers and in various sectors [9,10]. Compared to conventional technologies, microalgal cultivation is considered as an environmental friendly technology [11]. and having less impact on food supply and agricultural land [12–14]. In addition to carbon sequestration, microalgae also act as an efficient renewable sources towards fossil fuel replacement inspite of it's current limitations [13,15–17]. Microalgae possess certain advantages over terrestrial plants in terms of rapid growth rate, morphology, photosynthetic efficiency and higher CO_2 fixation efficiency [18–20].

CO_2 is majorly emitted from industrial sector (point source) or from transport sector (line source). Control and treatment of point sources of pollution is easier compared to line sources of pollution. The major point sources of CO_2 include the flue gas (15–20% CO_2 concentration) emitted from the thermal power plants and pure CO_2 (100%) emitted from the chemical industries such as distilleries. Micro algal technology could be used as a possible solution to control point source CO_2 emissions from chemical industries and thermal power plants. Number of research works have been carried out for controlling flue gas emission using microalgae. Table 1. gives the tolerance capability of various species for CO_2 concentration.

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Table 1
Tolerance capability of various microalgal species for varying CO_2 concentration.

Microalgal species	% of CO_2 concentration	Inference	Reference
Anabaena. CH1	5,10 and 15	Maximum biomass concentration and CO_2 fixation rate was obtained at 10% concentration of CO_2 which is 1.16 g/l and 1.01 g/L/day.	[21]
Scenedesmus obliquus SA1 (KC733762)	0.03–35%	Maximum biomass concentration was reported at 35% concentration of CO_2 and is 1.39 ± 0.023 g/l. CO_2 fixation rate 97.65 ± 1.03 mg $L^{-1} d^{-1}$.	[22]
Dunaliella tertiolecta SAD-13.86, Chlorella vulgaris LEB-104, Spirulina platensis LEB-52 and Botryococcus braunii SAG-30.81	Air sparged with 5% CO_2	Among the four microalgal species, *Botryococcus braunii* SAG-30.81 produced CO_2 fixation rate of 497 mg/L/day along with biomass productivity of 3.11 g/L.	[23]
Scenedesmus obliquus SJTU-3 and Chlorella pyrenoidosa SJTU-2	0.03–50%	Maximum biomass productivity of 1.84 ± 0.01 g/L and CO_2 fixation rate of 50.58 ± 0.08 was obtained at 10% CO_2 concentration for *Scenedesmus obliquus SJTU-3* and it decreased further with increasing CO_2 concentration.	[24]
Chlorella sp.	0–70%	Maximum growth of 5.772 g/L was reported at 10% CO_2 concentration. It decreased further with increasing CO_2 concentration. Microalgae was able to sustain till 70% CO_2 of 0.766 g/L concentration	[25]
Nannochloropsis sp. and Phaeodactylum sp.	Actual flue gas 10–12% CO_2, SOx and NOx are 70–90 ppm. Simulated flue gas only with 12% CO_2 and desulfured gas	Microalgae is able to sustain in the flue gas as equal to that of 12% CO_2 concentration in simulated flue gas.	[26]
Spirulina platensis	12% Actual desulfured flue gas from a power plant smokestack	Dry biomass of 1220Kg/year was achieved and it was 2.35 g/L.	[27]
Anabaena sp.	Flue gas	Maximum CO_2 fixation rate of 3.0 gCO_2 L^{-1} day^{-1} was observed in outdoors	[28]
Chlorella sp. MTF-15	Coke oven gas CO_2- 23–27% Hot Stove CO_2- 24–28% Power plant CO_2- 22–26%	Maximum biomass concentration of 0.515, 0.314 and 0.342 g/L/day for coke oven gas, hot stove and power plant respectively.	[29]
Spirulina platensis	Simulated diesel generator exhaust CO_2 11%; CO 1.5%	Maximum biomass concentration of 1.85 g/L was obtained on the sixth day.	[30]
Nannochloropsis sp. *Chlorella* sp. *Pseudochlorococcum* sp.	0.04–2% (v/v)	Maximum biomass concentration is 0.87 ± 0.05 g/L/day and CO_2 fixation rate is 1.538 g/L/day for Chlorella sp. in nitrogen sufficient conditions for *Chlorella* sp.in nitrogen sufficient conditions.	[31]
Chlorella sp. *Isochrysis* sp. *Amphidinium carterae*	10, 15 and 20% of flue gas CO_2 concentration	Maximum biomass productivity of 0.268 g/L/day and maximum CO_2 fixation rate of 0.492 g/l/day for *Chlorella* sp.	[32]
Synechococcus elongatus	Ambient air, 5% and 10% CO_2 concentration	Maximum biomass concentration is 2.1 g/L at 10% CO_2 concentration for membrane contactor PBR and 2.45 g/L at 5% CO_2 concentration for membrane sparger PBR.	[33]

The quantum of research works available on the tolerance capability of microalgal strains for pure CO_2 in to the medium is very less [25,34–37]. Other than the CO_2 concentration, a number of process parameters and environmental conditions such as, initial inoculum concentration [38], nutrients [39–41], light intensity [38,40], light to dark cycle ratio [38], pH [2,42,43], temperature [44] have influence on the microalgal growth and subsequently on secondary metabolites produced.

Diffusion capablity of CO_2 in water is 10,000 times slower than in air [45] and the solubility of CO_2 in water is also very less [46]. Hence when CO_2 is bubbled into the medium, there is a possibility of rapid release of CO_2 present in water to the atmosphere without significant biological utilization. Also during continuous supply of CO_2, pH is maintained at 5.5 which is highly unfavourable for microalgal growth. Due to above limitations continuous supply of CO2 into the system is not favourable for microalgal growth. Hence to improve the utilization of CO_2, it is purged in to the system intermittently having various supply frequency. This strategy may result in potential CO_2 mitigation through biological sequestration, by monitoring the availability of CO_2 in the medium. Also CO_2 is fixed by Ribulose −1,5-bisphosphate carboxylase oxygenase (Rubisco) through Calvin Cycle by the conversion of CO_2 to organic carbon. The maximum catalytic capacity of Rubisco can be achieved only when it is fully saturated with the substrate of CO_2 and ribulose-1,5-bisphosphate. Perhaps ambient CO_2 imparts only 25% catalytic capacity of Rubisco which may reduce the photosynthetic efficiency [22]. Hence to maintain the Rubsico in saturated form throughout the growth phase of the microbial system and to improve the CO_2 utilization in the medium, CO_2 could be sparged intermittently which might eventually increase the photosynthetic efficiency.

Also, addition of CO_2 into the medium is responsible for facilitating the exposure of cells to light and thereby minimizing the shading effect and avoids the dissolved oxygen accumulation in the system to reduce the toxic effects [47]. However, such addition may lead to the alteration of pH affecting the growth process. Hence, more studies are required to find out the growth rate and sustainability of species suitable for 100% CO_2 gas. To enhance the catalytic property of Rubisco enzyme, this study involves the growth and sustainability of *Oscillatoria* sp. supplied with pure CO_2 at various intervals and further to characterize the cultivated biomass under such conditions.

2. Materials and methods

2.1. Species and operating parameters

Oscillatoria sp. (Strain No: BDU 142191) was collected from the National Facility for Marine Cyanobacteria, Bharathidasan University, Tamil nadu. *Oscillatoria* sp. was cultivated in flat plate reactor having the dimensions of (33 × 22 × 10 cm) with a working volume of 4 L. The 12 h Light:Dark cycle photoperiod was maintained with a help of a timer. Four 36 W fluorescent lamps were used for illumination.The light intensity was maintained at 160 μE/m^2/s. It was measured using PAR Sensor (HydroLab DS 5, Make: Hach) and temperature was maintained at 25 °C.

Trials were made to cultivate the *Oscillatoria sp* .with the modified ASN III medium comprising only the major nutrients of the media such as sodium chloride, sodium nitrate, di potassium hydrogen phosphate and magnesium sulphate at varying concentrations. The culture was not able to survive in any of the modified ASN III media, hence experiments were continued with ASN III media [48] (sodium chloride- 25 g, magnesium chloride- 2.0 g, potassium chloride- 0.5 g, sodium nitrate-0.75 g, di-potassium hydrogen carbonate- 0.02 g, magnesium sulphate heptahydrate- 3.5 g, calcium chloride dihydrate- 0.5 g, citric acid- 0.003 g, ferric ammonium citrate- 0.003 g, EDTA disodium salt- 0.0005 g, sodium carbonate-0.02 g, trace metals- 1.0 ml per litre of water (Trace metal constituents; H_3BO_3- 2.86 g, $MnCl_2·4H_2O$- 1.81 g, $ZnSO_4·7H_2O$- 0.222 g, $NaMoO_4·2H_2O$- 0.39 g, $CuSO_4·5H_2O$- 0.079 g, $Cu(NO_3)_2$·6H2O- 49.4 mg in 1 l distilled water).

Pure CO_2 (100%) was admitted into the medium. Experiments were conducted by varying the supply frequency of CO_2 as once, twice and thrice per day during the light period. Control for the experiments, was the sample cultivated in ASN III media. The sample grown with 100% CO_2 gas supply for 15 min at 300 ml/min into the media, for once, twice and thrice are referred as medium F1, F2 and F3 respectively, where F denotes frequency. (The carbon source provided to microalgal system are the CO_2 supply and 20 mg/l of sodium carbonate in ASN III media.)

The initial culture concentration of all the reactors was kept at 0.243 g/l. The pH (a digital pH meter of model PH-98107 and make-HENNA™ Instruments) was measured before and after every supply of CO_2. Optical density was measured to monitor the growth of the species.

2.2. Growth and kinetic parameters

Optical cell density was measured as absorbance at 440 nm by spectrophotometric method using an UV/Visible Spectrophotometer (model Spectroquant®Pharo 300 and make-Merck). At the end of the batch experiment, microalgal biomass was harvested using centrifuge (Model R241of Make Remi PR-24) at the speed of 10000 rpm for 5 min. The pellets were washed with distilled water. The pellet was subjected for drying in a hot air oven (LabTech 2) at 60 °C till the dried biomass attained a constant weight (Sartorius BT 223S). The relationship between concentration (dry weight basis mg/l) and the optical density was obtained using a linear regression (Eq. (1))

$$Y = 0.7802X - 0.1953 \ (R^2 = 0.9975) \quad (1)$$

X is the optical density at 440 nm and Y is the dry biomass concentration (g/l).

Biomass productivity (g/l/d) was calculated using the following Equation.

$$P = (X_1 - X_0)/(t_1 - t_0) \quad (2)$$

X_1- Biomass concentration at time t_1 & X_o is the initial biomass concentration at time t_0.

Specific growth rate (μ,day^{-1}) was calculated using the following Equation.

$$\mu = \frac{Ln\left(\frac{X_1}{X_0}\right)}{t_1 - t_0} \quad (3)$$

Doubling time was calculated as follows:

$$td = \frac{Ln2}{\mu} \quad (4)$$

where, td is the doubling time.

2.3. CO_2 consumption, fixation, and removal

Total carbon of the sample was determined using CHN Series II 2400. According to the method described by de Morais and Costa 2007 [49], the carbon dioxide biofixation rate RCO_2 ($g\,L^{-1}\,d^{-1}$) was calculated using the following Equation:

$$Rco_2 = C_{carbon}P(Mco_2/M_c) \quad (5)$$

where C_{carbon} is the carbon content of the microalgal cell (%, w/w), P is the biomass productivity (g/l/d), M_C is the molecular weight of carbon and M_{CO2} is the molecular weight of CO_2.

CO_2 removal (%) efficiency was calculated by using the following Equation.

$$CO_2 \text{ removal } (\%) = \frac{\text{Total } CO_2 \text{ biofixed } (g)}{\text{Total } CO_2 \text{ input } (g)} \times 100 \quad (6)$$

CO_2 consumption rate C_{CO2} ($g\,L^{-1}\,d^{-1}$) at various frequencies of CO_2 supply was determined using the material balance as given in the following Equation.

$$C_{CO2} = P \times 1.87 \quad (7)$$

2.4. Growth kinetics using logistic equation

Logistic Equation holds good in describing the microbial growth that is independent of substrate concentration. Sigmoidal curve of Dry biomass vs time describe the microbial growth involving lag, log, stationary and death phase [50,51].

$$\frac{dx}{dt} = K_C X\left(1 - \frac{X}{X_{max}}\right) \quad (8)$$

Where X is the weight of the dry biomass (gL^{-1}), X_{max} is the maximum weight of dry biomass (gL^{-1}) and K_c is the apparent specific growth rate (day^{-1}). Upon integrating and rearranging Eq. (8), it can be rewritten as following:

$$X = \frac{X_{max}}{1 + \left(\frac{X_{max}}{X_0} - 1\right)e^{-k_c t}} \quad (9)$$

The above Equation can be further rewritten in the simplified form as following

$$X = \frac{a}{1 + be^{-K_c t}} \quad (10)$$

Where a is X_{max}, b is ((X_{max}/X_0) −1). Constants a and b were determined from the plot of dry biomass weight vs time in Origin Pro 8.0 using non-linear curve fitting tool with the confidence bound of 95%.

2.5. Biomass analysis

2.5.1. Ultimate analysis

The carbon, hydrogen and nitrogen contents were determined through a CHN Series II 2400. A sample ranging from 0.7 to 2 mg was weighed with a precision of 0.00001 mg (PerkinElmer Auto balance 6000 CE) in a tin foil cup. Oxygen gas at 18–20 psi was used for combustion of materials and helium gas 20 psi was used as a carrier gas for analysing the elemental composition.

2.5.2. Calorific value

The calorific value was determined using an automatic bomb calorimeter (IKA C5000) at adiabatic mode. Benzoic acid was used as a standard to calibrate the instrument. Dried algal samples were combusted in the bomb calorimeter to estimate the calorific value. Sample ranging from 0.8 to 1 g was weighed with a precision of 0.001 mg in a crucible (Sartorius BT223S). High purity oxygen (99.995%) was used for combustion.

3. Results and discussion

3.1. Effect of intermittent supply of CO_2 on pH

CO_2 is supplied during the light period of the cycle every day. CO_2 is available in different forms corresponding to the pH values, that is in the form of dissolved CO_2 gas (pH < 5), bicarbonate (7 < pH < 9) and carbonate (pH > 9) [52]. Every addition of CO_2 reduced the pH and subsequently the pH increased before the next supply of CO_2 (Fig. 1). Increase in the pH of the medium is the indication of growth of microalgae which occurs due to the uptake of CO_2 and bicarbonate [53–56] by the cells through the membrane releasing OH^- in the culture medium [57] and also due to the uptake of nitrates by the microalgae [58,59]. Basu et al. [60] also reported the increase in biomass concentration with increase in pH of the medium, which is similar to the results obtained by the present study.

The species was able to sustain the growth in the wide range of initial pH, ranging from 5.3 to 8.7 indicating that this species could be able to sequester more quantity of CO_2 unlike other species which hardly sustains in the acidic environment. Fig. 1 shows the microalgal culture grown under control, F1–F3. In control experiment, where CO_2 was not supplied, the pH got stabilised at 10.1 on the 14th day, indicating the growth got stabilised due to the unavailability of carbon source in the medium. Corresponding to medium F1–F3 the increased biomass growth was observed till 21 days (Fig. 1). Variation in pH was maximum for higher supply frequency of CO_2 (Medium F3).

Fig. 2 exhibits the plot of delta pH vs specific growth rate cultivated under Media F1–F3. Delta pH is the variation of pH observed for every 24 h. In all the three cases increase in delta pH is associated with corresponding increase in specific growth rate. Maximum specific growth rate occurs on the second day of the growth phase for all the three cases. Fig. 2 leads to inference that increase in pH has the direct influence on specific growth rate.

Similar correlation between pH and growth rate was found for the *Nannochloropsis salina* by Bartley et al. [2]. Delta pH observed on the first day is maximum for all the three cases and it is found to be 4.2, 2.9 and 2.3 for medium F1–F3 respectively. Subsequently delta pH is reduced in the growth phase of the system, and it is lying in the range of 2.5–2.8 for medium F1 and 1.4–1.5 for medium F2 and medium F3. Delta pH is calculated on daily basis. Delta pH is less for medium F2 and F3 compared to F1, since over the same period the CO_2 was supplied twice and thrice respectively.

Intermittent supply of CO_2 was chosen to maximise the carbon utilisation and to stabilise the required pH of the process. Continuous supply of CO_2 at any concentration will reduce the pH and acidic atmosphere is lethal to microalgae growth [61]. Supply frequency of CO_2 needs to be optimised corresponding to the type of species and environmental conditions.

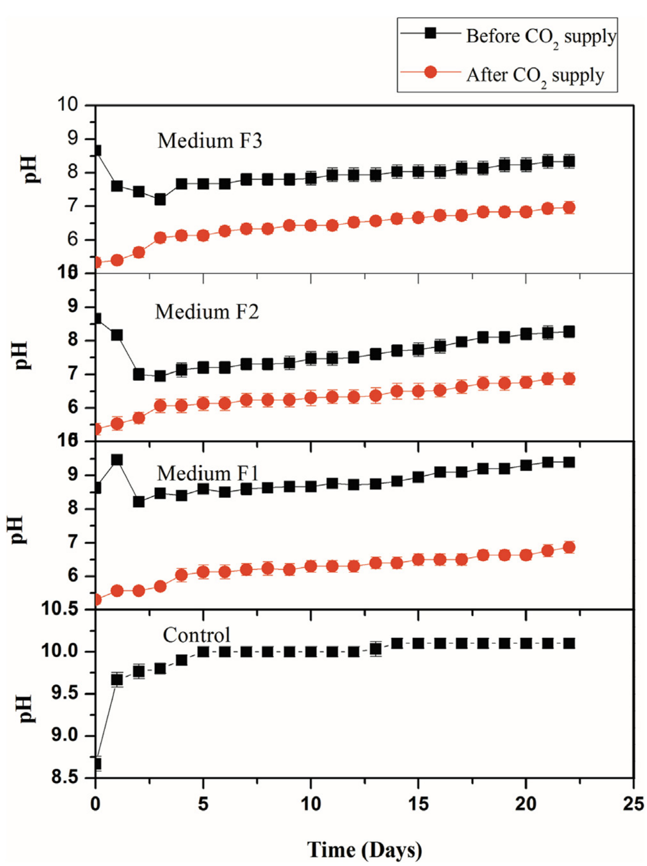

Fig. 1. Variation of pH before and after CO_2 supply in control, Medium F1–F3 for *Oscillatoria* sp. Error bars denote standard deviations for n = 3.

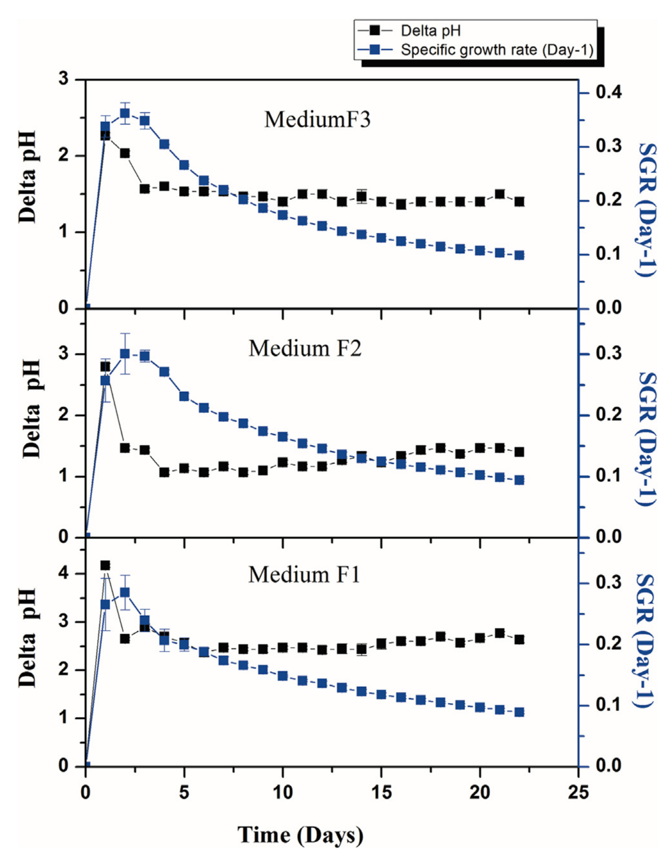

Fig. 2. Variation of delta pH and Specific growth rate with Time for *Oscillatoria* sp. grown in Medium F1,F2 and F3. Error bars denote standard deviations for n = 3.

3.2. Effect of supply frequency of CO_2 on the kinetic parameters of *Oscillatoria sp.*

The batch cultivation period of *Oscillatoria* sp. grown in various medium was 22 days. The species could not grow in the modified ASN III media containing only the major salts such as NaCl, $NaNO_3$, K_2HPO_4 and $MgSO_4$ at different reduced concentrations than that in the ASN III media. The majority of the nutrients of ASN III are available in sea water, except nitrate. (Composition of salts as Chloride 19345 ppm, Sodium 10752, Magnesium 1295 ppm, Sulphate 2701 ppm, Potassium 390 ppm, Calcium 416 ppm, Bromide 66 ppm [62]. However addition of nitrate is essential for the sp. but the overall cost of nutrient requirements is much less, considering sea water for cultivation. Variation of biomass concentration with time for the batch period corresponding to the various supply frequencies of CO_2 is shown in Fig. 3. The values of various kinetic parameters with respect to the different media are reported in Table 2. *Oscillatoria* sp. cultivated under media F1, produced the biomass yield of 1.67 ± 0.10 g/l with the maximum specific growth rate of 0.284 ± 0.04 day^{-1} which was comparable to the results of Sydney et al. who reported the biomass yield of 1.94 g/l for the *Chlorella sp* cultivated under 5% CO_2 with the maximum specific growth rate of 0.29 day^{-1} [23]. Increased biomass productivity was reported at 35% CO_2 concentration compared to lower concentrations for *Scenedemus obliquus* SA1 [22]. The reported productivity of *Scenedemus obliquus* SA1 is 51.94 ± 0.5 mg/lit/day and is less compared to the productivity of *Oscillatoria* sp. observed for media F1–F3 of the present work. Bhaktha et al. [63] studied the consortium isolated from waste water treatment plant having *Chlorella* sp. *Scenedesmus* sp. *Sphaerocystis* sp., *Spirulina* sp., exhibited maximum growth at 20% CO_2 concentration, the culture expressed higher growth up to 50%. However at 100% CO_2 concentration, growth was inhibited. Therefore *Oscillatoria* sp. did not only exhibit the better tolerance to higher percentage of CO_2 but also resulted in appreciable increase in biomass productivity.

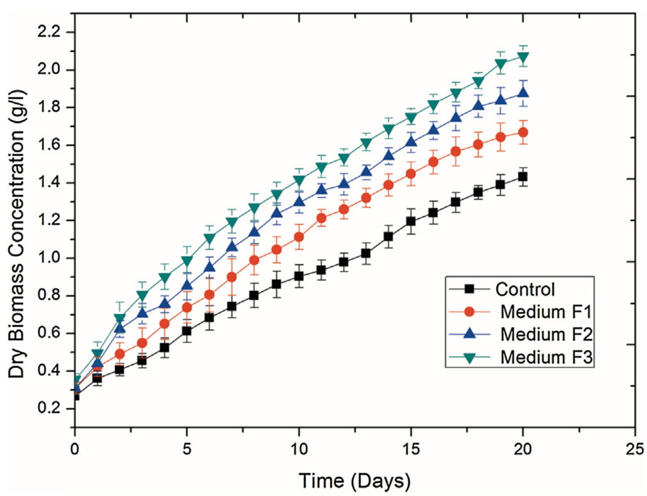

Fig. 3. Effect of varying supply frequency of CO_2 on biomass concentration of *Oscillatoria* sp. Error bar represents SD for n = 3.

Oscillatoria sp. showed a considerable increase in growth with an increased supply frequency of pure CO_2 (Table 2). It was 1.45 times for Medium F3, 1.24 times for Medium F2 and 1.1 times for Medium F1 compared with the control. Similar observation was reported by Sutherland et al. on the frequency of pure CO_2 supply upon the treatment of domestic waste water. They found that the addition of CO_2 for every 15 min, produced 120% higher organic biomass yield compared to low frequency of CO_2 addition given at 90 min time interval [64]. Table 3. shows the biomass productivity of *Chlorella* sp. and *Oscillatoria* sp. at different flow rates of CO_2. It could be inferred from Table 2 that the *Oscillatoria* sp. produces higher biomass yield at a higher flow rate of CO_2, indicating that this species is capable of fixing more carbon. Also specific growth rate with time was highest for medium F3 throughout the batch period.

Logistic model was used for fitting the growth data obtained from various frequencies of CO_2 supply (Table 4). Biomass concentration predicted from the model, for the medium F2 & F3 were very close to the experimental data and the variation was less than 5% (Table 4 and Fig. 4). Deviation from the experimental data was found to be less than 10% for all the media. Curve fitting of the model with experimental data was appreciably good having R^2 = 0.97–0.99. However the apparent specific growth rate (K_c) obtained from the model was lying between 0.167 to 0.20 day^{-1} and is approximately twice that of the net specific growth rate (Table 4). Similar variation between the apparent specific growth rate (K_c) and net specific growth rate was reported by Kumar et al. [51].

Table 2
Growth parameters of *Oscillatoria* sp. cultivated in various media.

Oscillatoria sp. grown in	Maximum Biomass (X_{max}) g/l	Maximum Biomass productivity *P max* mg/l/day	μmax (day^{-1})	μnet (day^{-1})	Doubling time (day)
Control	1.43 ± 0.08	0.361 ± 0.06	0.20 ± 0.02	0.085 ± 0.004	8.133 ± 0.375
Medium F1	1.67 ± 0.10	0.422 ± 0.08	0.284 ± 0.04	0.092 ± 0.003	7.49 ± 0.3
Medium F2	1.87 ± 0.11	0.44 ± 0.10	0.3 ± 0.03	0.098 ± 0.004	7.07 ± 0.312
Medium F3	2.07 ± 0.09	0.495 ± 0.10	0.361 ± 0.03	0.102 ± 0.004	6.75 ± 0.30

The data are given as mean ± SD, n = 3.

Table 3
Comparison of Biomass productivity of microalgal species at pure CO_2 (100%).

Microalgal species studied	Flow rate (ml/min)	Biomass productivity (mg/litre/day)	Reference
Chlorella sp.	20	30.51	[43]
	50	41.40	
	100	23.28	
Oscillatoria sp. Medium F1 (Once supply frequency)	300	75 ± 0.005	This study
Medium F2 (Twice supply frequency)	300	80 ± 0.005	
Medium F3 (Thrice supply frequency)	300	92 ± 0.004	

Table 4
Logistic model exhibiting initial and final biomass concentratin and apparent specific growth rate with R^2 values pertaining to various frequencies of CO_2 supply.

Oscillatoria sp grown in	$X_0(gL^{-1})$	X_{max} (gL^{-1})	Kc (d^{-1})	R^2
Control	0.287	1.57	0.167	0.98
Medium F1	0.302	1.76	0.200	0.99
Medium F2	0.354	1.96	0.200	0.98
Medium F3	0.422	2.14	0.191	0.97

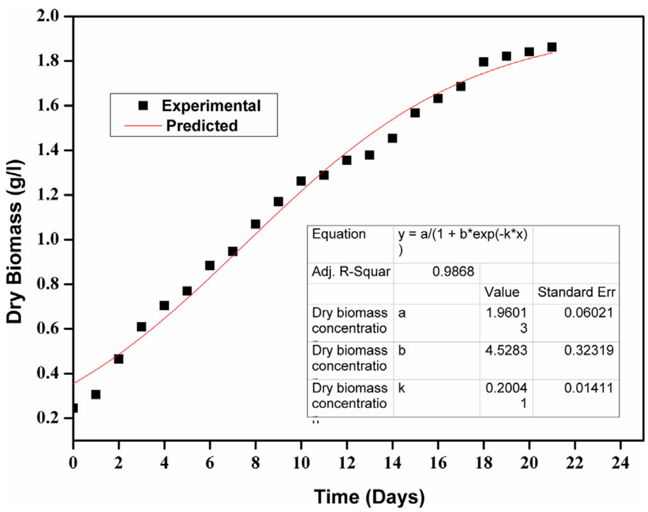

Fig. 4. Simulated growth profile of *Oscillatoria* sp. cultivated in Medium F2 using Logistic model.

3.3. Effect of CO_2 fixation, utilization and removal

CO_2 fixation of microalgae in the reactor depends upon the carbon assimilation ability of a microalgal species. Microalgae have inorganic carbon (Ci) transporters along with the cell membrane (Ci) transporters in the chloroplast where the photosynthesis occurs. CO_2 or bicarbonate imported in to the cell was present mainly as bicarbonate in the chloroplast stroma. Bicarbonate is taken to the thylakoid lumen converting to CO_2 by the carbonic anhydrase enzyme. Due to increased concentration of CO_2 in the thylakoid lumen, CO_2 is transported to the pyrenoid matrix through pyrenoid tubules in the thylakoid membrane. CO_2 is converted into organic carbon by ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) in the Calvin cycle [65]. The enhanced fixation rate observed with increased supply frequency of CO_2, is due to the fact that the catalytic capacity of Rubisco is increased by the concentration of CO_2 as well as with the supply frequency.

CO_2 could be made available for micro algal growth in the form of dissolved CO_2 by a continuous supply of CO_2. Continuous supply of CO_2 has two disadvantages: 1) Solubility of CO_2 is limited in the medium, 2) fixation rate of CO_2 by algae is limited. Hence the excess CO_2 gets into the atmosphere without being utilised by the cells. It also leads to acidic pH which may not be suitable for microalgal growth. In this study, supplying the CO_2 intermittently gave a better impact on the carbon fixation rate of the microalgal species.

CO_2 fixation and CO_2 removal by the *Oscillatoria* sp. are shown in Fig. 5. It shows that the CO_2 fixation increased with the supply frequency of CO_2. Fixation rate was increased from 116 ± 0.007 in medium F1 to 156 ± 0.004 mg/l/day in medium F3. Tang et al. reported that the CO_2 fixation rate is maximum at a concentration of 10% for *Chlorella pyrenoidosa* and *Spirulina obliquus*. Reduced CO_2 fixation rate was observed at 50% concentration, and it was 108 ± 0.007 and 105 ± 0.006 mg/l/day for *Chlorella pyrenoidosa* and *Spirulina obliquus* [24] which is lower than the present study conducted with pure CO_2. Anjos et al. studied the CO_2 fixation rate of *Chlorella vulgaris* as a function of aeration rate (0.1,0.4 and 0.7 vvm) and CO_2 concentration (2, 6 and 10%) and reported a maximum carbon fixation rate of 2.22 g/litre/day corresponding to a 6% concentration of CO_2 and 0.4 vvm of aeration rate [4]. Hence, to maximize the CO_2 fixation of any microalgal species, the concentration, flow rate and supply frequency of CO_2 need to be considered together.

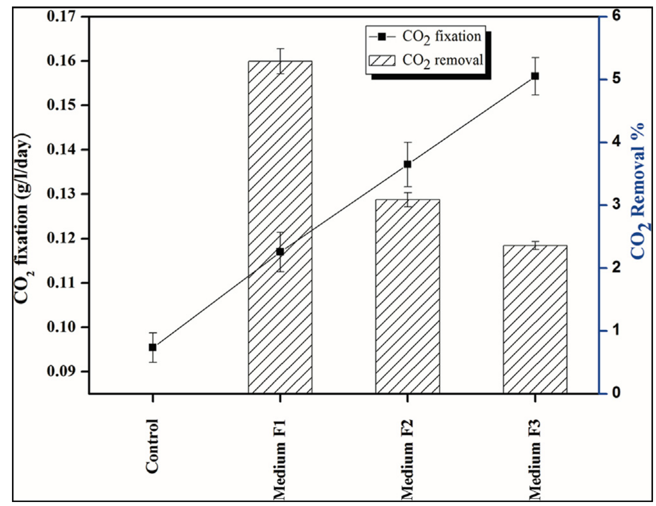

Fig. 5. Effect of varying supply frequency on CO_2 fixation and CO_2 removal of *Oscillatoria* sp. Error bar represents SD for n = 3.

The effect of CO_2 concentration on carbon removal efficiency was studied by Lam et al. and reported that the carbon removal efficiency gradually decreased with the increased concentration of CO_2 [66]. It is due to the limitation of CO_2 solubility in the media. Carbon removal efficiency could be improved by supplying CO_2 intermittently, only to an extent of the saturation limit attained by the medium, instead in a continuous manner. CO_2 removal efficiency of *Oscillatoria* sp. decreased as the supply frequency of CO_2 increased (Fig. 5). This indicates that the CO_2 supplied was much higher than the saturation limit. CO_2 removal efficiency could be increased by supplying CO_2 only till the medium reaches

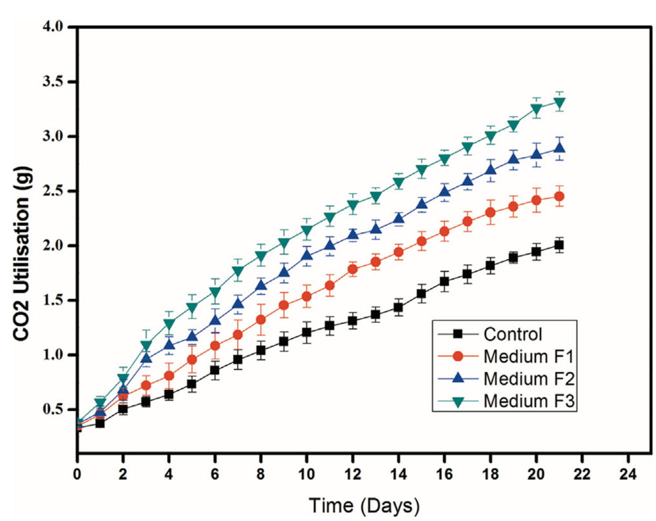

Fig. 6. Effect of varying supply frequency on CO_2 utilisation of *Oscillatoria* sp. Error bar represents SD for n = 3.

Table 5
Elemental analysis and calorific value of algal biomass in various media.

Oscillatoria sp. grown in	C (%w/w)	H(%w/w)	N(%w/w)	O(%w/w)	Empirical formula	Molecular Weight (g)	Calorific value
Control	38.45 ± 0.04	6.77 ± 0.03	6.12 ± 0.02	48.64 ± 0.09	$CH_{2.11}N_{0.137}O_{0.95}$	31.22	14.63 ± 0.45
Medium F1	40.20 ± 0.03	6.97 ± 0.05	7.24 ± 0.04	45.58 ± 0.12	$CH_{2.08}N_{0.16}O_{0.85}$	29.92	16.17 ± 0.34
Medium F2	41.88 ± 0.03	7.24 ± 0.03	7.75 ± 0.03	43.11 ± 0.10	$CH_{2.07}N_{0.16}O_{0.77}$	28.63	16.56 ± 0.39
Medium F3	43.42 ± 0.03	7.78 ± 0.03	8.44 ± 0.02	40.35 ± 0.04	$CH_{2.15}N_{0.16}O_{0.69}$	27.43	17.21 ± 0.29

The data are given as mean ± SD for n = 3.

saturation, increasing the mass transfer area, and increasing the residence time of CO_2.

Utilization of CO_2 at various supply frequencies are shown in Fig. 6. Carbon utilization of *Oscillatoria* sp. was derived from the mass balance of photosynthesis reaction by stoichiometric analysis. Carbon utilization increased as the frequency of CO_2 supply increased. Maximum CO_2 utilization in medium F3 was 3.317 ± 0.15 g, which was 1.65 times higher than the control. Hence the efficiency of photosynthesis is also increased by 1.65 times which may be due to the saturation level attained by Rubisco with CO_2.

3.4. Effect of CO_2 on calorific value and carbon content

Carbon,Hydrogen and Nitrogen of the *Oscillatoria* sp. at different frequencies of 100% CO_2 supply were estimated using CHN analysis and reported in Table 5. Molecular formula of *Oscillatoria* sp. was arrived by using the weight percent of estimated CHN content. Molecular weight of *Oscillatoria* sp . ranges from 27.43 to 31.22 g which is similar to the results obtained in Kumar et al. [67]. Highest molecular weight of 31.22 g was obtained for *Oscillatoria* sp. grown as control. Stoichiometric analysis provides the quantity of CO_2 consumed and O_2 released for the production of 1 g of biomass. Stoichiometric equations at various supply frequencies of CO_2 are given below.

$$\text{Air (Control)} + 1.055\, H_2O + 0.137\, NO_3 \rightarrow CH_{2.11}N_{0.137}O_{0.95} + 1.258\, O_2$$

$$CO_2\ \text{(Media F1)} + 1.04\, H_2O + 0.16\, NO_3 \rightarrow CH_{2.08}N_{0.16}O_{0.85} + 1.34\, O_2$$

$$CO_2\ \text{(Media F2)} + 1.035\, H_2O + 0.16\, NO_3 \rightarrow CH_{2.07}N_{0.16}O_{0.77} + 1.38\, O_2$$

$$CO_2\ \text{(Media F3)} + 1.075\, H_2O + 0.16\, NO_3 \rightarrow CH_{2.07}N_{0.16}O_{0.77} + 1.43\, O_2$$

CO_2 uptake and O_2 liberation is increasing with the supply frequency of CO_2, indicating that the *Oscillatoria* sp. is tolerant to pure CO_2. Production of 1 g of biomass consumes 1.60 g of CO_2 and liberates 1.43 g of O_2 for the *Oscillatoria* sp. cultivated in Media F3. C, H and N values follow the increasing trend with the increase in frequency of CO_2 supply. The calorific value of the microalgae usually ranges from 3500 to 4500 cal/g [68] and similar range of calorific values are attained in this study. Calorific value of *Oscillatoria* sp. for control was 14.63 ± 0.45 MJ/kg whereas for Medium F1–F3 it was slightly increased to 16.17 ± 0.34 MJ/Kg, 16.56 ± 0.39 MJ/Kg,17.21 ± 0.29 MJ/kg respectively and the corresponding carbon content were 38.45 ± 0.04%, 40.20 ± 0.03%, 41.88 ± 0.03% and 43.42 ± 0.03%. Increase in the calorific value with respect to the supply frequency is due to the increase in the contents of C, H and N values. External supply of CO_2 simultaneously enhanced the CO_2 sequestration by microalgae while increasing the calorific value of the biomass, which clearly proves the assimilating capability of the species for gaseous CO_2. However, the calorific values attained are lower than the reported value of 18.59 ± 0.42 MJ/kg by Phukan et al. for *Chlorella* sp. MP-1 [69]. Lower content of calorific value in *Oscillatoria* sp. is due to lower carbon content than the compared microalgal species. Screening of microalgal species with the high carbon content and the assimilating capacity of CO_2, may facilitate the improvement in calorific value as well as lipid content.

4. Conclusions

Supply frequency of CO_2 has a positive impact on growth of biomass, carbon fixation and energy content. *Oscillatoria* sp. could be the suitable species to mitigate pure CO_2, since biomass yield was increased by 44.7% in presence of CO_2, than the control conditions. Optimisation studies by varying the flow rates and supply frequency of CO_2 during the light cycle will be helpful for increasing the biomass yield. Screening of algae with reference to the pure CO_2 tolerance and energy content, could lead to sequester CO_2 from distilleries emitting 100% CO_2 during the fermentation process.

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