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Full Length Research Paper

Carbon sequestration potential of *Scenedesmus species* (Microalgae) under the fresh water ecosystem

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The study was conducted to investigate the carbon sequestration potential of *Scenedesmus* species (a microalgae species) under the fresh water ecosystem. Biofixation of CO_2 by microalgae mass cultures represents an advanced, climate friendly biological process that enables the direct utilization of fossil CO_2 streams produced from concentrated sources, such as power plants. The study was done in Nagpur region, the sample of *Scenedesmus* species (a microalgae species) were collected from fresh water ponds scattered over in Nagpur area. Collected samples were isolated for *Scenedesmus* species with Streak plate and Pour plate method by using serial dilution, purification was done by antibiotic treatment. The growth of *Scenedesmus* species was tested under different concentration of CO_2, heavy metal treatment and different pH values and also chlorophyll contents were estimated over the various concentrations of CO_2. Results revealed that the highest growth reported at 36% of CO_2 treatment, 7.4 to 8.0 pH values and growth inhibited under high concentration of heavy metals. Chlorophyll synthesis was reported highest at 36% of CO_2 treatment. With the results it concluded that the *Scenedesmus* species having good carbon sequestration potential as it can tolerate up to 48% CO_2 concentration in the medium, and can give excellent biomass accumulation under the 36% CO_2 contents in the medium. This *Scenedesmus* species can be exploited as good source of bio-fixation of environmental CO_2.

Key words: *Scenedesmus* species, biofixation, carbon sequestration, microalgae.

INTRODUCTION

Climate change is caused by natural internal process or external forces or by persistent anthropogenic changes in the composition of the atmosphere or in land use. Carbon dioxide is the principal greenhouse gas. Atmospheric CO_2 has increased from 280 to 368 ppm in the last 200 years and is responsible for about 50% enhancement in the greenhouse effect (Karube et al., 1992). Global warming is induced by the increased concentration of carbon dioxide (CO_2) in the atmosphere and estimated to induce severe impacts on human activity in the near future (McCarthy et al., 2001). The carbon cycle involves the whole biosphere, as carbon moves through the air, rocks, soil, water and all living things in a cyclical process. All life is dependent on this process and carbon serve as the principal elements of which all living beings are made (Sandquist and Ehleringer, 1995). The part of carbon is absorbed and contained in nonliving forms such as oceans, glaciers and rocks which serve as sinks, which helps in the accumulation of CO_2 in the atmosphere. The carbon dioxide fixation rates are associated with energy received by the cells during the light periods. Maximum carbon dioxide fixation rates of 1.440 g/L per day were found for cultures with a continuous supply of light energy. A linear reduction in the CO_2 fixation rates with the reduction in duration of the light period was evident, with the exception of the 12:12 (night:day) cycles. Biological fixation of carbon dioxide is an attractive option because plants naturally capture and use carbon dioxide as a part of the photosynthetic process.

Bio-fixation of CO_2 using photosynthetic organisms has been looked at as a way to stop or slow down the effects of global warming. Since the creation of carbon sinks on land using plants would be of such high cost and require a large amount of land, the use of algae has been seen

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as a more feasible solution. Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, but due to a simple cellular structure and submerged in an aqueous environment where they have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass. Microalgae are a group of unicellular or simple multi-cellular photosynthetic microorganisms that can fix CO_2 efficiently from different sources, including the atmosphere, industrial exhaust gases and soluble carbonate salts. The most frequently used micro-algae are *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including the diatoms) and *Chrysophyceae* (including golden algae). It is generally assumed that those species that exhibit the highest maximal specific growth rate will also have the highest biomass productivity that is, the best CO_2 bio-fixation potential (Eppley and Dyer, 1965). Highly CO_2-tolerant microalgae and cyanobacteria for biological fixation of CO_2 such as *Anacystis*, *Botryococcus*, *Chlamydomonas*, *Chlorella*, *Emiliania*, *Monoraphidium*, *Rhodobacter*, *Scenedesmus*, *Spirulina* and *Synechococcus* (Sawayama et al., 1995; Sung et al., 1999). Growth medium must provide sufficient nutrients for micro-algal growth.

Carbon, nitrogen, phosphorus, and sulfur are the most important elements constituting algal cells and other essential elements including iron, magnesium, trace elements, in some cases silicon (Fuentes et al., 2001). Cyanobacteria have the unique characteristic of using CO_2 in the air as a carbon source and solar energy as an energy source. Reducing equivalents from the fermentation of carbohydrates are used as the primary electron donors in Cyanobacteria for the hydrogen producing enzymes. The cells take up CO_2 first to produce cellular substances, which are subsequently used for H_2. A CO_2 uptake rate per unit cell decreased linearly with the initial CO_2 concentration in the gas phase. With repeated injections of CO_2, the CO_2 was continuously consumed and the cell concentration reached 3.7 g dry cell/L in 20 days, which is 6.7 times higher than that in a batch culture without further supply of CO_2. The CO_2 injection in the cell growth phase increased not only the cell concentration but also the hydrogen production per gram cell (Park et al., 2001). The goal of this study is to isolate microalgae in lakes and ponds which can tolerate high CO_2 concentrations and high temperatures in order to bio-fix carbon dioxide and discover the optimal conditions for biomass production.

MATERIALS AND METHODS

Chemicals and media

Analytical grade chemicals (Merck, Germany) were used for the preparation of media and heavy metal treatment solution.

Source of isolates

Microalgae (*Scenedesmus sp.*) were isolated from different lakes and ponds in Nagpur region. In addition to physical properties (pH, color and light intensity), of water, lakes and ponds were also determined.

Culture medium

Medium BG-11 contained (g/L): $NaNO_3$, 1.5; $K_2HPO_4.3H_2O$, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02 and trace metal solution of 1 ml (including H_3BO_3 2.86 g, $MnCl_2.4H_2O$ 1.81 g, $ZnSO_4.7H_2O$ 0.222 g, $Na_2MoO_4.2H_2O$ 0.390 g, $CuSO_4.5H_2O$-79 mg and $Co(NO_3)_2.6H_2O$ 49.4 mg/L) at pH 7.4 (Rippka et al., 1979).

Isolation of carbon dioxide fixing microalgae

Samples were precultivated in an appropriate broth for 1 week and sub cultivated for another week, culture broth was smeared on different solid media and cultivated at 30°C for 1 week. Colonies were picked and transferred to the same media for purification. 180 conical flasks were taken and 20 conical flasks were used as a control (without CO_2) and 160 conical flasks were used as a sample (with CO_2). The inoculation was carried out before two or three days of passing CO_2 concentration (12, 24, 36 and 48%). In each conical flask, different percentage of CO_2 concentration was passed by bubbling method for 60 s. In 60 s, 0.5 kg of CO_2 was passed with the help of flow cytometer. CO_2 cylinder was prepared in Aditya Air Product Private Limited, MIDC, Hingana Road, Nagpur. For the isolation of high CO_2 tolerant stains, the culture broth was aerated with 12, 24, 36 and 48% CO_2 at 30°C for 1 week.

Measurement of growth rate

The growth rate of microalgae was measured by optical density at 680 nm using UV-visible spectrophotometer (PG instruments, USA).

Effect of carbon dioxide on cell growth

Isolates were precultivated at 30°C in a 500 ml conical flask with 300 ml BG-11 medium and bubbled with air and air containing CO_2 for 20 days. Microalgae growth was determined by optical density at 680 nm.

Effects of heavy metals on inhibitory level

All algal forms were screened for zinc, cadmium, nickel, cobalt and copper toxicity using AR grade $ZnSO_4.7H_2O$, $3CdSO_4.8H_2O$, $NiCl_2.6H_2O$, $CO(NO_3)_2.6H_2O$ and $CuSO_4.5H_2O$.

Chlorophyll content

The chlorophyll content was estimated by the method of Liu et al. (1981). In which the chlorophyll was extracted by 95% ethanol and optical density was measured at 649, 655 and 665 by using UV-visible spectrophotometer (PG instruments, USA). The chlorophyll a and b content was calculated by the following equations:

C_a (µg ml^{-1}) = 13.7 A665 nm - 5.76 A649 nm

Table 1. Effect of different concentration of CO_2 on *Scenedesmus* species growth.

CO_2 treatment	CO_2 treatment period (days)						
	0	2	4	6	8	10	12
Control	0.54±0.01	0.87±0.01	1.14±0.00	1.32±0.01	1.53±0.05	2.02±0.01	2.12±0.00
12% CO_2	0.65±0.01	1.19±0.00	1.22±0.01	1.31±0.06	1.82±0.01	1.87±0.01	1.95±0.07
24% CO_2	0.94±0.01	1.01±0.00	1.22±0.01	1.55±0.01	1.55±0.01	1.82±0.01	1.86±0.01
36% CO_2	0.75±0.01	1.38±0.05	1.37±0.01	1.51±0.00	1.76±0.01	2.14±0.01	2.24±0.01
48% CO_2	0.84±0.01	1.19±0.01	1.59±0.01	1.58±0.01	2.01±0.00	2.02±0.01	2.19±0.01

Absorbance (optical density) was taken from Spectro photometrically at 680 nm. Age of the algae: 10 days old.

Table 2. Effect of different concentration of pH on growth of *Scenedesmus* species.

pH treatment	pH treatment period (days)						
	0	2	4	6	8	10	12
6.5	0.54±0.01	0.87±0.01	1.14±0.00	1.32±0.01	1.53±0.02	2.02±0.01	2.10±0.00
7.0	0.65±0.01	1.19±0.00	1.22±0.01	1.31±0.06	1.82±0.01	1.87±0.01	1.95±0.07
7.4	0.94±0.01	1.01±0.00	1.22±0.01	1.55±0.01	1.82±0.01	1.89±0.01	2.15±0.01
8.0	0.75±0.01	1.38±0.05	1.37±0.01	1.51±0.00	1.76±0.01	2.14±0.01	2.24±0.01
8.5	0.84±0.01	1.19±0.01	1.59±0.01	1.58±0.01	2.01±0.00	2.02±0.01	2.11±0.01

Absorbance (optical density) was taken from Spectro photometrically at 680 nm. Age of the algae: 10 days old.

C_b (µg ml^{-1}) = 25.8 A649 nm - 7.60 A665 nm

C_{a+b} (µg ml^{-1}) = 1000/39.8 × A655 nm)

Treatment of pH

The growth of algae essentially depends upon H-ion concentration of the medium. Therefore, a series of experiments was performed to study growth of *Scenedesmus* species with pH ranging from 7.0 to 8.0, the pH was adjusted by 0.1 NaOH/HCl. The experiment was carried out for 20 days.

RESULTS

Effect of CO_2 concentration on cell growth (*Scenedesmus* species)

The biomass concentration values measured as optical density (OD) at 680 nm for *Scenedesmus* species growing in the presence of four different concentrations of CO_2 that is, 12, 24, 36 and 48%. OD was higher when *Scenedesmus* species grown under 36% CO_2 concentration. The maximum OD (2.24±0.01) was in 36% CO_2 concentration and minimum (0.65±0.01) in 12% CO_2 concentrations (Table 1). The optical density was high in all four CO_2 concentrations as compare to control.

Effect of pH on the growth

It was observed that the growth of *Scenedesmus* species at pH 7.4 showed marked increase in the growth, indicating the alkaline pH is necessary for growth of the micro-algae (Table 2).

Effect of heavy metals on the growth

The heavy metals are present in natural water resources and they have inhibitory effect on the growth of micro-algae. Treatment results revealed that the zinc (0.120±0.002 mg/L), cadmium (0.023±0.002 mg/L), copper (0.035±0.003 mg/L), cobalt (0.015±0.002 mg/L) and nickel (0.020±0.004 mg/L) concentrations inhibited the growth of rate *Scenedesmus* species (Table 3).

Chlorophyll content under the treatment of different concentration of CO_2

Results revealed that the chlorophyll a (2.77±0.02 µg/ml), chlorophyll b (14.49±0.01 µg/ml) and chlorophyll a and b (11.93±0.01 µg/ml) contents were found highest at 36% CO_2 enriched culture as compared with the control (Table 4).

DISCUSSION

Effect of CO_2 concentration on cell growth (*Scenedesmus* species)

The biomass concentration values measured as optical density at 680 nm for *Scenedesmus* species growing in

Table 3. Effect of metals (inhibitory level) on growth of *Scenedesmus* species.

Metals (mg/L)	Inhibitory level
Cobalt	0.015±0.002
Cadmium	0.023±0.002
Copper	0.035±0.003
Nickel	0.020±0.004
Zinc	0.120±0.002

Absorbance (optical density) was taken from Spectro photometrically at 680 nm. Age of the algae: 10 days old.

Table 4. Effect of different concentration of CO_2 on chlorophyll contents of *Scenedesmus* species.

CO_2 treatment	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Chlorophyll (a+b) (µg/ml)
Control	0.53±0.01	8.64±0.03	7.42±0.03
12% CO_2	1.10±0.02	10.86±0.02	8.46±0.03
24% CO_2	1.61±0.01	8.76±0.01	7.73±0.01
36% CO_2	2.77±0.02	14.49±0.01	11.93±0.01
48% CO_2	1.54±0.01	12.15±0.01	10.74±0.01

Age of the algae: 10 days old.

the presence of four different concentrations of carbon dioxide that is, 12, 24, 36 and 48. In this present study on *Scenedesmus* species grown in media containing different CO_2 concentration (12, 24, 36 and 48%) shown remarkable growth at 36% CO_2 concentration. As it was reported that the HA-1 strain, identified as genus *Chlorella*, it showed maximum growth at 10% CO_2 enriched air flowing conditions, and showed a good growth rate in a broad range of physically controlled conditions. These results showed that *Chlorella* KR-1 is a promising strain to grow at extremely high CO_2 concentrations (Sung et al., 1999). Morais and Costa (2007) reported that the *Scenedesmus obliquus* were significantly lower at 28.08 and 13.56% when growing on 6 and 12% CO_2, respectively. When operating under optimum conditions, the capture efficiency has been shown to be as high as 99% (Zeiler et al., 1995). Ishida et al. (2000) reported that the cultures under bubbling of 10% CO_2 tolerant microalgae was isolated and identified as *Thalassiosira weissflogii* H1. There was no significant difference between the growth yields of this diatom under bubbling air, 5% CO_2 and 10% CO_2, but the growth yield under 20% CO_2 markedly decreased. The importance of biological mean of CO_2 fixation, more than 200 microalgae isolates were screened from lakes, ponds, sediments, hog wastewater, paddy fields, hot springs and seawater in Taiwan. Each liter of *Chlorella* sp. NTU-H15 produced 1.8 g of dry cell. While each liter of *Chlorella* sp. NTU-H25 produced 1.7 g dry cell.

The ZY-1 strain was identified as genus *Chlorella*. It showed maximum growth at 10% (v/v) CO_2 enriched air flowing condition, and a good growth rate in a broad range of physically controllable conditions, including CO_2 concentration up to 70% (v/v), CO_2 enriched air flow rate, temperature and pH (Yue and Chen, 2005).

Effect of heavy metals on the cell growth

In the present study, the inhibitory levels of zinc, cadmium, copper, cobalt and nickel on the growth rate of *Scenedesmus* species 0.120±0.002 mg/L zn, 0.023±0.002 mg/L cd, 0.035±0.003 mg/L cu, 0.015±0.002 mg/L co and 0.020±0.004 mg/L ni, these concentrations of heavy metals inhibited the growth rate of *Scenedesmus* species. As the study of Bartlett et al. (1974) showed that the treatment of metals 0.09 mg/L cu completely inhibited growth of *Selenastrum capricornutum* and 0.08 mg/L cd completely inhibited the growth of *S. capricornutum* while the nickel inhibited growth of *Scenedesmus* at 0.5 mg/L after a period of days, 25 mg/L zn exhibited pronounced toxicity for *Chlorella vulgaris* (Rana and Kumar, 1974).

Effect of pH on the growth

Different pH values were selected (6.5 to 8.5) for studying the maximum growth. It was observed that the algae at pH 7.4 to 8.0 showed marked increase in the growth indicating that alkaline pH is necessary for the growth of micro-algae (*Scenesmus* species). As it was reported that the intracellular pH value decreased from 7.0 to 6.4 when air-grown *Chlorococcum littorale* cells were exposed

to 40% CO_2 for 1 to 2 h, but noticeable decline was not observed. Both air and 5% CO_2 grown cells of *Chlorella* species UK001, which was also resistant to extremely high CO_2 concentrations grew in 40% CO_2 without any lag period. The present experiment findings indicate that the *Scenedesmus* species can be cultivated between pH ranges from 6.5 to 8.5.

Chlorophyll content under the treatment of different concentration of CO_2

Chlorophylls a (2.77±0.02 µg/ml) and b (14.49±0.01 µg/ml) counts were found highest at 36% CO_2 enriched culture as compared with the control followed by 48, 12 and 24% CO_2 enriched cultures. As the previous studies showed that the growth response of *C. vulgaris* was studied under varying concentrations of carbon dioxide (from 0.036 to 20%), temperature (30, 40 and 50°C). The highest chlorophyll concentration (11 µg/ml) and biomass (210 µg/ml), which were 60 and 20 times more than that of *C. vulgaris* at ambient CO_2 (0.036%), were recorded at 6% CO_2 level. At 16% CO_2 level, the concentrations of chlorophyll and biomass values were comparable to those at ambient CO_2 but further increase in the CO_2 level decreased both of them. *C. vulgaris* ARC-1 used in this study grew very well and showed significant gains in chlorophyll content and biomass up to 6% CO_2 level. It maintained the superiority over the ambient CO_2 level even when the CO_2 was raised up to 16% (Chinnasamy et al., 2009). When cells of *C. littorale* that had been grown in air were transferred to extremely high CO_2 concentrations (>20%), active photosynthesis resumed after a lag period which lasted for 1 to 4 days. In contrast, *C. littorale* cells which had been grown in 5% CO_2 (5% CO_2 grown cells) could grow in 40% CO_2 without any lag period.

When air grown cells were transferred to 40% CO_2, the quantum efficiency of PS II decreased greatly, while no decrease in PS II was apparent when the 5% CO_2 grown cells were transferred to 40% CO_2. In contrast to air grown cells, 5% CO_2 grown cells showed neither extracellular nor intracellular carbonic anhydrase activity. Upon the acclimation of 5% CO_2 grown cells to air, photosynthetic susceptibility to 40% CO_2 was induced, this change was associated with the induction of anhydrase activity.

Conclusion

The present investigation of the effect of CO_2 concentration on the growth of *Scenedesmus* species, under aeration condition with different concentration of CO_2 for 10 to 20 days shown that the *Scenedesmus* species grow slowly in air aeration condition. Treatment of different CO_2 concentration that is, 12, 24, 36 and 48% CO_2, showing that the CO_2 concentration is increasing the growth of the algae. The different concentration of pH also affect on the growth of *Scenedesmus* species, an initial pH was 7.4, increased gradually to 8.5. The cell growth of Scenedesmus species was inhibited at below 7.4, *Scenedesmus* species was not affected by cultured. The pH was higher than 7.4, the growth rate was high in present observation. The *Scenedesmus* species was able to grow at pH 7.4 to 8.5. All heavy metals are toxic to algae at high concentration. In this present study, *Scenedesmus* species growth was inhibited by the high level of heavy metals. All the heavy metals completely inhibited growth of algae at particular concentration.

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