

Carbon Sequestration by Blue Green Algae (Spirullina Species) Under the Fresh Water Ecosystem

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ABSTRACT

A typical Spirullina culture has been studied with the effect of different concentration of CO₂. The unisolated species were collected from fresh water ponds in Nagpur . Collected samples were isolated for Spirullina species with Streak plate and Pour plate method by using serial dilution, purification was done by antibiotic treatment. At different concentration of CO₂ and in different pH values the growth of Spirullina species was tested. This results concluded that the highest growth of Spirullina species was at 30% of CO₂ concentration, 7.4 to 8.0 pH values. At 30% of CO₂ concentration the highest growth of Spirullina species was reported . By this results it was estimated that the Spirullina species having good carbon sequestration potential as it can tolerate up to 40% CO₂ concentration in the medium, and can give excellent biomass accumulation under the 30% CO₂ contents in the medium. This Spirullina species can be exploited as good source of bio-fixation of environmental CO₂.

Key Words: sequestration, potential, bio-fixation

I. INTRODUCTION

The concentration of CO₂ in the air rose to 407ppm as recorded in 2018, from 200ppm in Ice Age, which is a direct consequence of industrialization. According to a study, the concentration of CO₂ would double and average temperature would rise by 1.5-3.0 degrees by the year 2030 if CO₂ continues to increase at the present pace. Carbon dioxide concentration increase in the atmosphere is associated to climate change and global warming.

The greenhouse gases (GHGs) cause depletion of ozone layer protecting the atmosphere against UV radiation, thereby warming the atmosphere. The average concentration of CO₂ increased from 315 ppm in 1960 to 380 ppm in 2007(IPCC, 2007). There has been a 35% increase in CO₂ emission worldwide since 1990. Carbon fixation by photoautotrophic algae has the potential to diminish the release of CO₂ into the atmosphere and in helping to alleviate the trend toward global warming.

Blue green algae are ubiquitous in nature. They occur in a wide variety of environmental conditions and wherever life is possible. Mainly The Cyanobacterian algae grow luxuriantly in the soil that's why the

occurrence and distribution is studied by number of phycologist throughout the world. Cyanobacteria are extraordinarily diverse group of Gram-negative, oxygenic photosynthetic prokaryotes that are distributed in all possible biotopes of the world. Due to their occurrence in diverse habitats, these organisms are excellent materials for investigation by ecologists, physiologists, biochemists, microbiologists.

The publications appeared during the second half of 20 th century have been confined to the blue green algae occurring within the soil. As blue green algae are productive in their nitrogen fixing ability and the nitrogen is the major requirement of paddy crop and the blue green algae are widely associated with this crop. Cyanobacteria also called blue green algae can fix CO₂ efficiently from many different sources, including the atmosphere, industrial exhaust gases and soluble carbonate salts. Cyanophyceae (blue-green algae), Chlorophyceae (green algae), Bacillariophyceae (including the diatoms) and Chrysophyceae (including golden algae) are the most frequently used micro-algae. Those species that exhibit the highest maximal specific growth rate will also have the highest biomass productivity that is, the best CO₂ bio-fixation potential was generally assumed (Eppley and Dyer, 1965).

Highly CO₂-tolerant microalgae and cyanobacteria for biological fixation of CO₂ such as *Anacystis*, *Botryococcus*, *Chlamydomonas*, *Chlorella*, *Emiliana*, *Monoraphidium*, *Rhodobacter*, *Scenedesmus*, *Spirulina* and *Synechococcus* (Sawayama et al., 1995; Sung et al., 1999). sufficient nutrients for micro-algal growth was provide from growth medium. Cyanobacteria i.e blue green alge have the ability to use CO₂ in the air as a carbon source and solar energy as an energy source. The cell concentration as well as the hydrogen production per gram cell will be increase by the CO₂ injection in the cell growth phase (Park et al., 2001). The aim of the current study is to isolate microalgae in lakes and ponds which can tolerate high CO₂ concentrations and high temperatures in order to bio-fix carbon dioxide and discover the optimal conditions for biomass production.

II. MATERIALS AND METHODS

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

Source of isolates: Microalgae (*Spirullina* sp.) were isolated from different lakes and ponds in Nagpur region. The physical properties (pH, color and light intensity), of water, lakes and ponds were also determined.

Culture medium: Medium BG-11 contained (g/L): NaNO₃, 1.5; K₂HPO₄·3H₂O, 0.04; MgSO₄·7H₂O, 0.075; CaCl₂·2H₂O, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na₂EDTA, 0.001; Na₂CO₃, 0.02 and trace metal solution of 1 ml (including H₃BO₃ 2.86 g, MnCl₂·4H₂O 1.81 g, ZnSO₄·7H₂O 0.222 g, Na₂MoO₄·2H₂O 0.390 g, CuSO₄·5H₂O-79 mg and Co(NO₃)₂·6H₂O 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. Picked up the colonies and transferred it to the same media for purification. 100 conical flasks were taken and 20 conical flasks were used as a control (without CO₂) and 80 conical flasks were used as a sample (with CO₂). Before two or three days of passing CO₂ concentration (10, 20, 30 and 40%), the inoculation was carried out. Different percentage of CO₂ concentration was passed in each conical flask by bubbling method for 60 s. In 60 s, 0.5 kg of CO₂ was passed with the help of flow cytometer. CO₂ cylinder was prepared in Aditya

Air Product Private Limited, MIDC, Hingana Road, Nagpur. For the isolation of high CO₂ tolerant stains, The culture broth was aerated with 10, 20, 30 and 40% CO₂ 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 CO₂ for 20 days. Microalgae growth was determined by optical density at 680 nm.

Table 1. Effect of different concentration of CO₂ on *Spirullina* species growth.

CO ₂ Treatment	CO ₂ treatment period(days)						
	0	2	4	6	8	10	12
Control	0.55±0.01	0.85±0.01	1.13±0.00	1.31±0.01	1.51±0.05	2.02±0.01	2.11±0.00
10% CO ₂	0.60±0.01	1.16±0.00	1.21±0.01	1.30±0.06	1.80±0.01	1.82±0.01	1.93±0.07
20% CO ₂	0.93±0.01	1.02±0.00	1.20±0.01	1.51±0.01	1.51±0.01	1.84±0.01	1.85±0.01
30% CO ₂	0.70±0.01	1.31±0.05	1.34±0.01	1.52±0.00	1.72±0.01	2.12±0.01	2.20±0.01
40% CO ₂	0.83±0.01	1.18±0.01	1.50±0.01	1.54±0.01	2.02±0.00	2.01±0.01	2.17±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 *Spirullina* species

pH treatment	pH treatment period(days)						
	0	2	4	6	8	10	12
6.4	0.52±0.01	0.85±0.01	1.12±0.00	1.31±0.01	1.52±0.02	2.01±0.01	2.11±0.00
7.0	0.63±0.01	1.15±0.00	1.20±0.01	1.30±0.06	1.80±0.01	1.83±0.01	1.93±0.07
7.4	0.91±0.01	1.01±0.00	1.21±0.01	1.51±0.01	1.81±0.01	1.82±0.01	2.12±0.01
8.0	0.73±0.01	1.36±0.05	1.35±0.01	1.50±0.00	1.74±0.01	2.12±0.01	2.20±0.01
8.4	0.86±0.01	1.16±0.01	1.54±0.01	1.53±0.01	2.00±0.00	2.01±0.01	2.10±0.01

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

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 *Spirullina* species with pH ranging from 6.4 to 8.4, the pH was adjusted by 0.1 NaOH/HCl. The experiment was carried out for 30 days.

III. RESULTS

Effect of CO₂ concentration on cell growth (*Spirullina* species)

The biomass concentration values measured as optical density (OD) at 680 nm for *Spirullina* species growing in the presence of four different concentrations of CO₂ that is, 10, 20, 30 and 40%. OD was higher when *Spirullina* species grown under 30% CO₂ concentration. The maximum OD (2.20±0.01) was in 30% CO₂

concentration and minimum (0.60 ± 0.01) in 10% CO₂ concentrations (Table 1). As compare to control the optical density was high in all four CO₂ concentrations.

Effect of pH on the growth

It was observed that the growth of *Spirullina* 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).

IV. DISCUSSION

Effect of CO₂ concentration on cell growth (*Spirullina* species)

CO₂ concentration (10, 20, 30 and 40%) shown remarkable growth at 30% CO₂ concentration. It was reported that maximum growth of HA-1 strain, identified as genus *Chlorella* at 10% CO₂ enriched air flowing conditions, and under a broad range of physically controlled conditions showed a good growth rate. This result conclude that *Chlorella* KR-1 is a promising strain to grow at extremely high CO₂ concentrations (Sung et al., 1999). The *Scenedesmus obliquus* were significantly showed lower growth i.e 28.08% and 13.56% when growing on 6 and 12% CO₂ according to Morais and Costa (2007). The capture efficiency has been shown to be as high as 99% when operating under optimum conditions (Zeiler et al., 1995). The cultures under bubbling of 10% CO₂ tolerant microalgae was isolated and identified as *Thalassiosira weissflogii* H1 was reported by Ishida et.al.(2000). The growth yield under 20% CO₂ markedly decreased but there was no significant difference between the growth yields of this diatom under bubbling air, 5% CO₂ and 10% CO₂.

Effect of pH on the growth

Different pH values were selected (6.4 to 8.4) 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 (*Spirullina* 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% CO₂ for 1 to 2 h, but noticeable decline was not observed. Both air and 5% CO₂ grown cells of *Chlorella* species UK001, It is resistant to extremely high CO₂ concentrations grew in 40% CO₂ without any lag period. *Spirullina* species can be cultivated between pH ranges from 6.4 to 8.4, This was the finding of the experiment..

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VI. REFERENCES

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