Bio-Fixation of Carbon Dioxide using Carbonic Anhydrase through Synthesis of Bicarbonate

Amol Deshmane[1], Yogesh Patil[2], Ravikant Langade[2], Deepali Nimbalkar[3] and Vikram Ghole[4]

Scientist, Department of Environmental Sciences, Vasantdada Sugar Institute, Pune, Maharashtra, India[1]

Research Student, Department of Environmental Sciences, Vasantdada Sugar Institute, Pune, Maharashtra, India[2]

Senior Scientist & Head, Department of Environmental Sciences, Vasantdada Sugar Institute, Pune, Maharashtra, India[3]

Coordinator, Academic Cell, National Institute of Virology, Pune, Maharashtra, India[4]

ABSTRACT: Carbon dioxide is a green house gas, responsible for potential threat of global climate change. Reduction and stabilization of such green house gas concentration is of utmost importance. Photosynthesis in green plants achieves natural carbon dioxide fixation with algae having higher photosynthetic potential than terrestrial plants. A biocatalyst in the form of carbonic anhydrase enzyme was used in the experiment in order to fix the carbon dioxide. The catalyst was supplied externally in modified Zarrouk media which was without sodium bicarbonate. This enzyme efficiently converted carbon dioxide into bicarbonate at ambient temperature and pressure producing 3,072mg/L and 27,805mg/L of bicarbonate 30 and 240 seconds respectively. *Spirulina* was released in the flasks containing modified Zarrouk media with bicarbonate synthesized by the activity of externally supplied carbonic anhydrase. The growth of *Spirulina* was observed for 120h and found comparable with the growth observed in standard Zarrouk media.

KEY WORDS: Carbon dioxide fixation, Carbonic anhydrase, *Spirulina*, bicarbonate synthesis

I. INTRODUCTION

Development in the current world is largely dependent on use of fossil fuels for energy generation (e.g. coal based thermal power plants) as well as for other industrial, automobile, and household activities. The combustion of fossil fuels is responsible for 73% of the carbon dioxide production [1, 2, 3] and fossil fuels account for 87% of the primary energy consumption [4]. Carbon dioxide is a green house gas and the potential threat of global climate change has been mainly attributed to greenhouse gas emissions from fossil fuel usage [5]). Therefore, compatible mitigation strategies are required to neutralize the excess carbon dioxide [6]). Carbon sequestration, capturing and storing carbon emitted from the global energy system, is a major tool for reducing atmospheric carbon dioxide emissions.

Photosynthesis in green plants achieves natural carbon dioxide fixation. It is estimated that natural processes remove CO_2 only about 12 Gtonnes of carbon dioxide. Numerous microalgae have a superior productivity compared to the other terrestrial plants under proper conditions of light, temperature, nutrients, available carbon dioxide concentration [7,8, 9]. Therefore, in recent years, microalgae have attracted a great deal of attention for bio-fuel production carbon dioxide fixation and wastewater treatment, as they spontaneously convert carbon dioxide and supplementary nutrients into biomass in the presence of light via photosynthesis, and at much higher rates/efficiency than conventional oil-producing crops [10]. This high efficiency is primarily due to the action of carbonic anhydrase (CA) enzyme, both extracellular and intracellular, and the carbon dioxide concentrating mechanisms (CCM) [11, 12,13]

The combination of algal carbon dioxide fixation, wastewater treatment and biofuel production has been investigated by a number of researchers (Tam and Wong, 2000 [14]; Guzzon et al. 2008 [15]; Kumar et al. 2010 [16]). However, the major technical challenge for microalgae based bio-fixation of carbon dioxide is the high production cost,

particularly the nutrient media and carbon cost. Therefore, there is a need for the cost reduction in the overall process. It is also essential for maintaining environmental and economic sustainability [17, 18,19]. The sequestration cost can be reduced, if the carbon dioxide sources are cheaper (such as emissions from industry) and captured efficiently. Carbonic anhydrase catalyses a reversible reaction and rapidly converts carbon dioxide into bicarbonate (HCO_3). It is a broad group of zinc metalloenzymes that is ubiquitous in nature [20,21,22].

In the present paper, experiments were conducted for carbon dioxide fixation in *Spirulina* species, using carbonic anhydrase for efficient capture of carbon dioxide and its conversion into bicarbonate.

II. MATERIALS AND METHODS

2.1 Materials

a. Carbon dioxide gas cylinder (purchased from local vendor); the gas was supplied to experimental set up through a regulator operating at low pressure; (GCE[Gmbh] DruVa MAKE FMD Prior Double-stage, Inlet Pressure : 230 Bar (Max) Outlet Pressure 0.1 to 4 bar (1.5 to 60 PSI).

b. Zarrouk media: It is well known for the cultivation of *Spirulina* (Zarrouk, 1966 [23]). Regular standard media as well as modified media were used for the experiment in the following way.

- Modified Zarrouk media, containing all constituents same as regular media except sodium bicarbonate was used in experimental flasks and control A
- Zarrouk media used as a standard media to grow *Spirulina* in control B set of experiment. This control was used to compare growth of *Spirulina* of experimental set.

c. ***Spirulina* culture:** Pure culture of *Spirulina* species was obtained from *Krishi Vidnynan Kendra* (Agriculture Science Centre), located at Babhaleshwar, District Ahmednagar, Maharashtra state, India.

d. Erythrocytes: Erythrocytes (Red Blood Cells) of goat blood were used as a source of CA enzyme. Fresh blood of goat was obtained from slaughter houses, and anti-coagulant (sodium oxalate) was added. Erythrocytes (RBCs) were isolated by centrifugation at 4,000rpm; washed thrice, re-suspended in normal saline (0.9% NaCl) and suitably diluted to get optical density 0.952 (± 0.005) of diluted cell suspension at 510nm. CA is a metallo-enzyme, found in many organisms, and contains one atom of zinc essential for activity in at its active site[24[25]) reported an enhancement of CA activity by erythrocytic membranes; CA I and CA II activity were increased 1.6 and 3.5-fold, respectively, by the presence of red cell membranes. Hence, the entire cells (erythrocytes) were used as an enzyme source.

e. Spectrophotometer (Hach make, model R 2000)

f. Digital thermometer

g. Lux meter (Lutron make)

2.2 Methods:

- **Bicarbonate estimation:** Two indicator method – It is divided into two parts; 1st part determination of alkalinity as per Standard methods by APHA 21st Edition [26] and 2nd part –as per Gran Titration [27]

2.2.1 Experimental Set up

The first part of the experiment was focused on carbon dioxide gas into bicarbonate in experimental flask containing modified Zarrouk media using CA as a biocatalyst (used in the form of erythrocytes). In the second part of the experiment, *Spirulina* was released in the flasks containing modified Zarrouk media and it was observed for five days for its growth. The growth of *Spirulina* was measured and compared with the growth in standard Zarrouk media. The entire experiment was performed at ambient temperature, maximum 32^{0}C ($\pm 2^o$C) minimum 20^{0}C ($\pm 2^o$C) and pressure. The experiment was performed in three replicate cycles/batches.

While designing the experiment two control sets were used.

- Control A: Only 200ml modified Zarrouk media was used without adding erythrocytes, prepared to check activity of CA present in erythrocytes for experimental flasks 1-8

- Control B: In this flask, 200ml Zarrouk medium was used. This was prepared to compare the growth of *Spirulina* from experimental flasks

For the first part of experiment, nine borosilicate conical flasks were used, of which one was used as control. In eight experimental and one control A flask, 200ml of modified Zarrouk media (i.e. without sodium bicarbonate) was taken and 5ml Erythrocytes were added to the experimental flasks. Carbon dioxide gas was supplied to all the flasks at 0.2bar pressure, in ascending time manner i.e. 30 seconds for flask 1, 60seconds for flask two and up to 240 seconds for eighth flask (table 1). When carbon dioxide was passed through the medium, bicarbonate was produced. It was estimated by double titration method immediately after carbon dioxide supply was stopped.

In the second part of the experiment, Control B flask containing 200ml standard Zarrouk media was added to the set. In all experimental flaks as well as control A and B 50ml of pre-washed *Spirulina* culture was added. Consistency in *Spirulina* cell density for all flasks was maintained by keeping optical density 0.60($\pm$0.010) at 660nm. After this, all flasks were maintained in the laboratory at ambient temperature, pressure for five days, to monitor the growth of *Spirulina.* A cycle of 12h light/12h dark was maintained in the laboratory. Light intensity was 750 to 1,500 Lux. Cell density of *Spirulina* was measured for all the flasks at 24h interval (table 2). Dry biomass was recorded at initial stage and after120 hours. It was determined by filtering sample (50 ml) through Whatman filter paper No.41, washing with distilled water and drying residue at 60°C in oven till the constant weight was obtained.

Figure 1: Experimental setup with cylinder supplying carbon dioxide to the experiment flasks

III. RESULTS AND DISCUSSION

The focus of first part of the experiment was to check the efficiency of CA enzyme provided in the medium for rapid conversion of carbon dioxide into bicarbonate (HCO3-), which is a stable and environmentally safe. It was assumed that, the bicarbonate thus produced, would act as a source of inorganic carbon (Ci) for *Spirulina*. An extensive literature survey was done before designing this experimental setup/process.

Alava et al. [28] reported that if bicarbonate is used as carbon source, it represents 60% of the cost of nutrients for cultivation of algae. For autotrophic growth (which is more suitable for large-scale open cultivation of algae) carbon can be provided as carbon dioxide, carbonate or bicarbonate. In microalgae, the extracellular CA occurs in the plasma membrane and converts bicarbonate^{-} to carbon dioxide, which is a planar and apolar molecule that can pass freely through the lipid bilayer of algal cellular membrane as reported by Axelsson et al. [29]. Internally, CA may be located in the cytoplasm, mitochondria and mainly in the chloroplast [30]. In the cytoplasm, the internal enzyme acts converting carbon dioxide $_2$ into bicarbonate - to prevent the leaking of carbon dioxide from the cell [31, 32]. In the chloroplast, bicarbonate is converted to carbon dioxide by another CA, concentrating carbon dioxide around RUBISCO, helping to overcome the low affinity that this enzyme has for carbon dioxide [33]. Regardless of the species, it is bicarbonate taken up from the external medium, that is delivered at the inner side of the plasma

membrane. Here specifically located CA generates carbon dioxide in the immediate vicinity of Rubisco [34, 35,36]. This is a part of the carbon dioxide Concentrating Mechanism (CCM) that is found mainly in microalgae and cyanobacteria [37, 38].

The results of the experiment show that, bicarbonate synthesis was in a very small amount i.e. 500mg/L observed in the control A, whereas extensive synthesis of bicarbonate was observed for flasks 1-8, provided with external CA enzyme (table 1). Therefore, it was quite evident that, bicarbonate synthesis in these flasks was due to CA supplied to the medium.

Table 1: Bicarbonate synthesis observed in the experiment

Flask #	1	2	3	4	5	6	7	8	Control A
Flask contents	200 ml modified Zarrouk media (i.e. without sodium bicarbonate) containing 50ml *Spirulina* culture and 5ml erythrocytes (RBCs) as a source of carbonic anhydrase (CA)								Same as flasks 1-8, but no erythrocytes were added
CO_2 supply duration (seconds)	30	60	90	120	150	180	210	240	120
Bicarbonate content (mg/L) at the end of first part of experiment	3,072 (±25.42)	6,453 (±24.13)	14,823 (±25.17)	21,340 (±36.05)	24,828 (±25.53)	26,140 (±40)	27,333 (±28.87)	27,805 (±8.66)	502 (±4.01)

Figures in parenthesis denotes standard deviation

The results of second part of the experiment related to increase in the cell density, show that bicarbonate served as a source of carbon for the growth of *Spirulina*. This was evident from decrease in the bicarbonate content at the end of 120h as well as growth of *Spirulina* measured in terms of increase in the cell density as well as dry biomass. More interesting results were observed for experimental flasks 5 to 8 (Table 2). Here, the *Spirulina* growth was quite comparable to control B (Standard Zarrouk media). Bicarbonate utilization for control B set was 1,086mg L^{-1} d^{-1}. Whereas, bicarbonate utilization was for flask 2 to 8 was ranging from 1,149mg L^{-1} d^{-1} for flask 2 upto 1,461 mg L^{-1} d^{-1} for flask 8 (Figure 1). Kaplan & Reinhold [39,40] reported that cyanobacteria can actively take up bicarbonate as a dissolved inorganic carbon (DIC) species from the medium. The bicarbonate pumps afford intracellular concentrations up to three orders of magnitudes higher than those in the external medium. This could be the reason of higher utilization of bicarbonate by *Spirulina*.
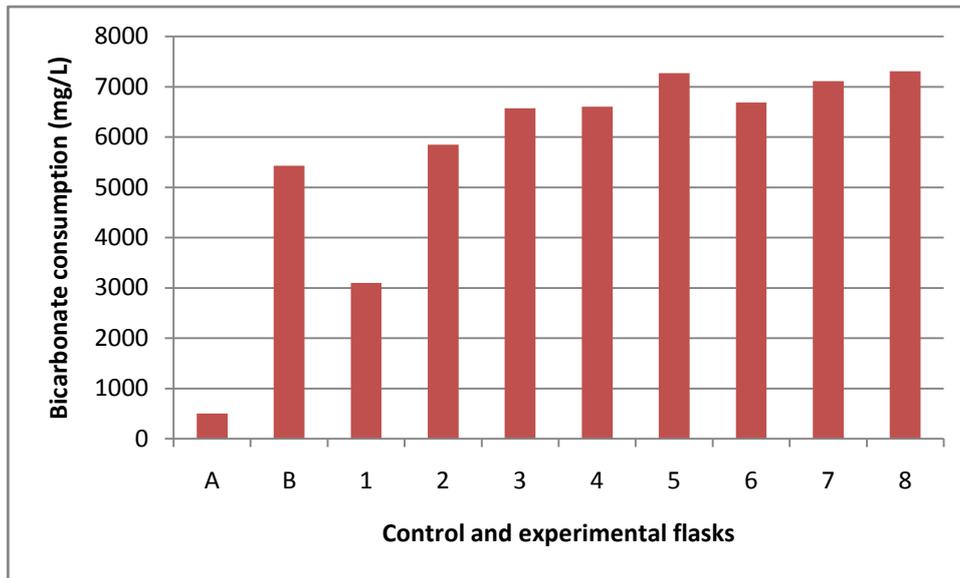
Figure 1: Bicarbonate consumption after 120h (five days) by *Spirulina*

Table 2: Change in the cell density of *Spirulina* after completion of the bicarbonate synthesis reaction over the period of 120h

| Flask	Duration of passing CO2 (seconds)	Cell density measured at 660nm wavelength					
		Initial	24h	48h	72h	96h	120h
1	30	0.230 (± 0.001)	0.302 (±0.006)	0.369 (±0.006)	0.395 (±0.007)	0.404 (±0.006)	0.368 (±0.003)
2	60	0.230 (±0.001)	0.303 (±0.007)	0.371 (±0.006)	0.431 (±0.007)	0.538 (±0.006)	0.585 (± 0.005)
3	90	0.230 (±0.001)	0.307 (±0.007)	0.378 (±0.007)	0.473 (±0.008)	0.550 (±0.006)	0.665 (±0.007)
4	120	0.230 (±0.001)	0.309 (±0.007)	0.374 (±0.009)	0.474 (±0.008)	0.565 (±0.006)	0.685 (±0.007)
5	150	0.230 (±0.001)	0.311 (±0.006)	0.389 (±0.017)	0.480 (±0.008)	0.572 (±0.006)	0.713 (±0.006)
6	180	0.230 (±0.001)	0.312 (±0.005)	0.398 (±0.009)	0.484 (±0.007)	0.576 (±0.006)	0.720 (± 0.008)
7	210	0.230 (±0.001)	0.313 (±0.006)	0.401 (±0.010)	0.487 (±0.007)	0.579 (±0.006)	0.727 (±0.006)

8	240	0.230 (±0.001)	0.318 (±0.010)	0.404 (±0.013)	0.509 (±0.007)	0.598 (±0.009)	0.763 (±0.010)
Control A	120	0.230 (±0.001)	0.241 (±0.030)	0.257 (±0.024)	0.272 (±0.019)	0.295 (±0.027)	0.302 (±0.006)
Control B	No CO2 passed (Zarrouk media)	0.230 (±0.001)	0.371 (±0.005)	0.428 (±0.005)	0.528 (±0.005)	0. 652 (±0.008)	0.835 (± 0.005)

Figures inparenthesis denotes standard deviation

Overall, the growth of *Spirulina* in experimental flasks 5-8 was 0.44 to 0.47g d^{-1} per 50ml (dry weight basis), where as in control B flask growth rate was observed to be 0.65g d^{-1} per 50ml (Figure 2). Jime´nez et al. [41] extrapolated an annual dry weight biomass production rate of 30 tonnes per hectare using data from a 450 sq. m and 0.30 m deep raceway pond system producing biomass dry weight of 8.2 g m^{-2} per day in Malaga, Spain. Using similar depth of culture, and biomass concentrations of up to 1 g l^{-1}, Becker [42] estimated dry biomass productivity in the range of 10–25 g m^{-2} per day.
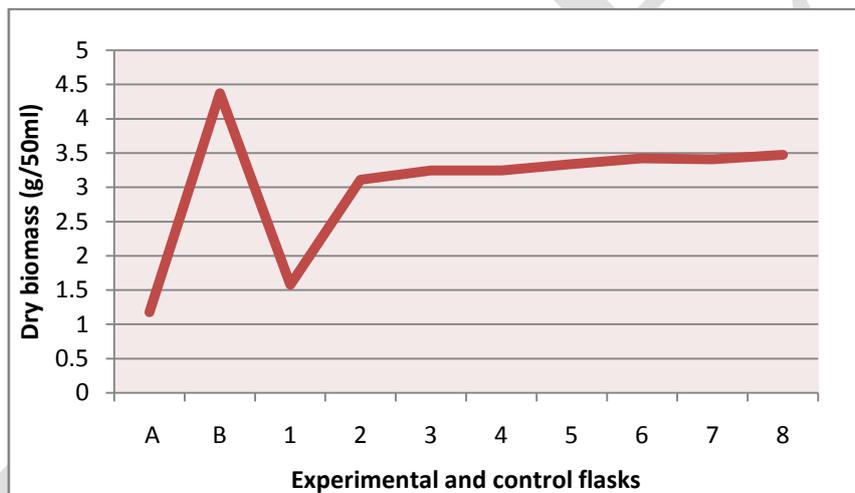
Figure 2: Dry biomass of *Spirulina* (g per 50ml sample) for control A and B as well as experimental flasks

IV. CONCLUSION

Rapid conversion of carbon dioxide into bicarbonate was achieved by the catalytic activity of provided CA enzyme. The bicarbonate produced was used by *Spirulina* as a source of carbon. Thus, the research provides strong clue to overcome the constraint of cost of carbon source (mainly $NaHCO_3$) for large scale cultivation of *Spirulina* (or any algae). This could be beneficial for cultivation of algae for the production of bio-fuels and vast array of its applications. Further, it can be inferred that the technology could prove useful for mitigation of carbon dioxide from various industrial point sources, such as fermentation units of distilleries, carbon dioxide from bio-digesters, etc. The technology with some modifications can be useful to sequester the carbon dioxide from flue gases of thermal power plants. Since, the process takes place at ambient temperature and pressure, energy saving is presumed to be another major advantage of the technology. Therefore, a cheaper and environment friendly option of carbon dioxide mitigation is emerging through this research.

V. ACKNOWLEDGEMENT

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