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Experimental Measurement of Total Inorganic Carbon Concentrations from Absorption of Gas Phase CO_2

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Abstract: Sodium hydroxide solution was mixed with cyanobacteria growth media (50% RO water and 50% BG – 11) in a ratio of 1 mL of 3M NaOH per liter a of media (3mM/L) to examine the effects on CO_2 solubility using air (0.038%), 0.1%, 0.5%, 1.0%, 1.5%, 2.0% and 10% CO_2 under atmospheric pressure at temperatures of 25, 35, 45, and 55˚C. In addition, the solubility of 0.5% and 1.0% CO_2-enriched air was investigated its behavior with respect to changing of NaOH concentration. The NaOH solution played a significant role for CO_2 solubility in increasing CO_2 solubility holding capacity. Ratios of TIC response among 0.1%, 0.5%, 1.0%, 1.5%, and 2.0% CO_2 solubility to NaOH were approximately 1 and 2 for 10.0% CO_2 compared to 0.1%, 0.5%, 1.0%, 1.5%, and 2.0% CO_2,which was much less than the theoretical driving force ratio of these CO_2 levels. These ratios decreased with respect to increased temperature. The TIC from CO_2 solubility was linearly proportional to the amount of the NaOH solution used and to the increased CO_2 levels. Experimental results yielded meaningful correlations of TIC concentrations and a bulk parameter as a function of temperatures, CO_2 concentrations and amounts of the NaOH concentration.

Keywords: carbon dioxide; CO_2; themophilic cyanobacteria; cyanobacteria growth media; RO water; inorganic carbon; saturated TIC concentration; total inorganic carbon; TIC.

I. INTRODUCTION

Total inorganic carbon (TIC) concentration from the absorption of gaseous CO_2 in defined algal growth media as a function of temperature and gas phase CO_2 concentration is a major carbon source for aquatic photosynthetic organisms. Further, it is known that gas phase CO_2 levels affect algal growth, but not in an easily predictable manner. Ono and Cuello reported that microalgae can grow under a variety of CO_2 concentrations from ambient air (0.04%) to 100% by-volume and in temperatures ranging from 25 to 100˚C [1]. Although cyanobacteria could grow in a wide range of CO_2 concentrations, Miyairi stated that productivity of cyanobacteria at 0.3% gas phase CO_2 concentration significantly increased compared to ambient air [2]. However, little change in productivity was found when CO_2 concentrations between 0.3% CO_2 and 5% CO_2 provided insignificant difference in biomass productivity [2]. In addition, a 60% CO_2 concentration reduced the productivity compared to 0.04% and 0.3% CO_2.

Temperature affects the solubility of CO_2. At high temperatures and gas phase CO_2 concentrations, the concentration of inorganic carbon may be so low as to limit the photosynthetic process [3–4]. In addition, inorganic carbon species vary with pH, as shown in Figure 1 [5–7]. This is important, because the carbon concentrating mechanism of aquatic species may prefer different inorganic carbon species. Improper growth pH may limit availability of the preferred inorganic carbon species and thus impact photosynthesis [7-8].

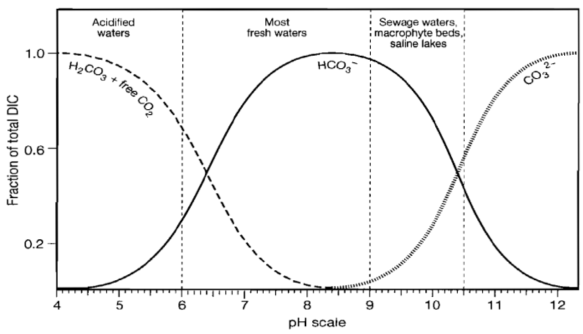

Fig. 1. Distribution of inorganic carbon forms in rivers and lakes with respect to changes in pH [3]

Most aquatic species do not directly use CO_2 from the air in a gas phase for the process of photosynthesis, but instead use an aqueous form of inorganic carbon; therefore, the formation of inorganic carbon from gas phase CO_2 absorption is important to algal growth. Gaseous CO_2 dissolves in water as shown in Equation (1), which obeys Henry's law. The dissolved carbon dioxide

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($CO_2(aq)$) then reacts with water to form carbonic acid, as shown in Equation (2). Then, $H_2CO_3^*$ illustrating a combination of aqueous CO_2 and carbonic acid dissolves in water as shown in Equations (3) and (4), which are much faster reactions than Equations (1) and (2) [5].

$$CO_2(g) \leftrightarrow CO_2(aq) \quad (1)$$

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3(aq) \quad (2)$$

$$H_2CO_3^*(aq) \leftrightarrow HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad (4)$$

Aqueous CO_2 (or carbonic acid) predominates when the pH is below 6.3, HCO_3^- predominates at $6.3 \le pH \le 10.3$, and CO_3^{2-} is the major species at $pH \ge 10.3$ [3, 6].
In theory, TIC concentrations can be predicted from CO_2 concentration and media composition. However, compared to our experimental measurement of TIC concentration, most existing models are lacking. For example, the predicted TIC levels from Carroll et al. and PHREEQC for a gas phase CO_2 concentration of 0.5% in water and actual TIC measurement are compared as shown in Figure 2.

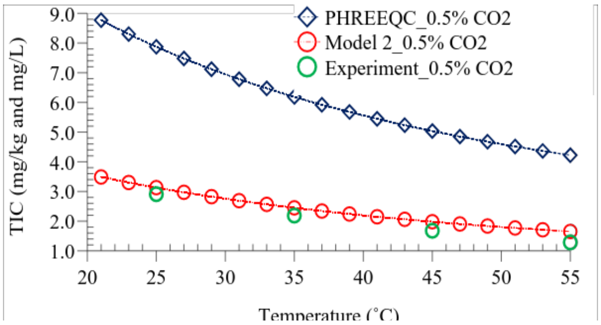

Fig.2. TIC concentrations from a gas phase CO_2 concentration of 0.5% in RO water from PHREEQC, model 2 (Carroll et al.) and experimentation

The TIC concentrations predicted from PHREEQC and the Carroll model were higher than the actual measured values. These results and similar ones indicated a strong need to create a comprehensive database of TIC concentrations as a function of temperature and media composition for future modeling efforts.

II. EXPERIMENTAL SETUP

Total inorganic carbon concentration was measured at experimental conditions including temperatures of 25, 35, 45, and 55 ˚C and gas phase CO_2 concentrations of air (380 ppmv), 0.5%, 1.0%, 1.5%, 2% and 10.0% in reverse osmosis (RO) water and growth media (GM), RO with 3 mM NaOH (RO+) and GM with 3 mM NaOH (GM+). A gas phase concentration of 0.1% CO_2 was also used to investigate the TIC concentration in GM+. 3 M NaOH solution at a 1:1000 dilution was added to RO and GM increase the CO_2 absorption capacity, creating the RO+ and GM+, respectively. GM was a mixture of 50:50 by volume between BG-11 and RO water. The BG-11 composition was shown in Table 1. Finally, each TIC measurement was replicated three times (from the same sample) to improve data reliability.

Table 1 Composition of BG-11

Chemicals	g/L-RO water	g/RO water	
		15 gal	25 gal
1. $NaNO_3$	0.500	28.500	47.500
2. K_2HPO_4	0.040	2.280	3.800
3. $MgSO_4$	0.075	4.275	7.125
4. $CaCl_2$	0.036	2.052	3.420
5. Citric Acid	0.006	0.342	0.570
6. Fe ammonium citrate	0.006	0.042	0.570
7. EDTA	0.001	0.057	0.095
8. Na_2CO_3	0.002	1.140	1.900

Two experimental setups, an Erlenmeyer flask and recirculating falling-films, were used to experimentally measure TIC concentrations. Schematic diagrams for the Erlenmeyer flask and recirculating falling-film setups are shown in Figures 3 and 4, respectively.

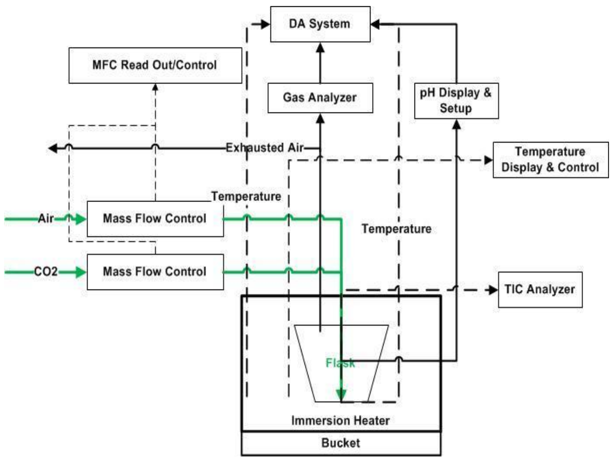

Fig.3. Schematic diagram for bubbling CO_2 in an Erlenmeyer flask

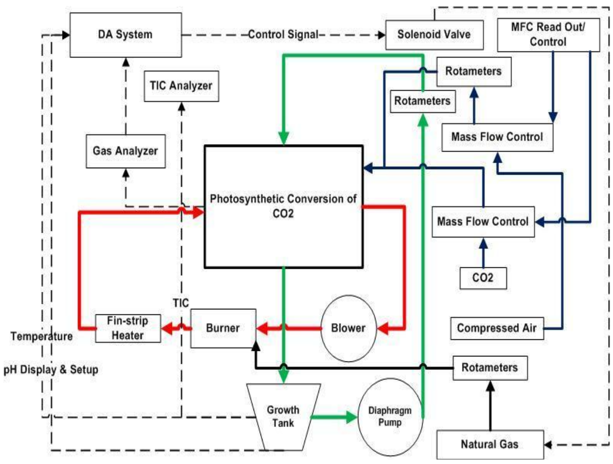

Fig. 4. Schematic diagram for bioreactor CO_2 delivery and measurement system

A. Data Acquisition System
A Campbell Scientific CR1000 data acquisition system was used for continuous display and automatic recording of temperature, pH, and CO_2 gas phase concentrations. Application of the system for measurement of specific parameters is described in the sections of this chapter specific to those parameters.

B. pH Measurement
The pH of the growth media varies inversely with inorganic carbon concentration and affects speciation; thus, it is necessary to record the media pH over the same time as TIC measurement. A combination of a Thermo Scientific submersible automated temperature compensated pH probe, with a temperature range of -10.0 to 110.0 ˚C with a resolution and accuracy of 0.1 ˚C and ±0.5 ˚C, and its display and read out, Model Alpha pH500 2-Wire pH/ORT transmitter pH/mV/°C, which displays pH from 0.00 to 14.00 with resolution and accuracy of 0.01 pH and ±0.01 pH, were employed for continuous pH measurement. A 4-20 mA output from the display was wired to the data acquisition system for continuous recording.

C. CO_2-enriched Gas Delivery System
To achieve the desired gas phase concentration of CO_2, Omega mass flow controller models FMA-7110 and FMA-7102, were used to control the flow of CO_2 and air respectively. The read-out and control module, FMA-5876A, was used to set the flow rates of the mass flow controllers (MFCs).

A Nova gas analyzer, Model WP-375, was employed to measure and display the gas phase CO_2 concentration. The Nova gas analyzer is able to detect CO_2, O_2, and CO in the ranges of 0-20%, 0-25%, and 0-2000 PPM, respectively. This analyzer was calibrated before each use using a GTS Welco gas mixture: 9.98% CO_2, 101 PPM CO and N_2 balance with analytical accuracy of ±2% full scale for CO_2 and CO. Accuracy and repeatability of this Nova gas analyzer are ±2% full scale for CO_2 and O_2, and ±10 PPM CO. A 4-20 mA output from the gas analyzer was wired to the data acquisition.

The experiments with a very low CO_2 concentration (< 0.1% CO_2) were in the lower end of the gas analyzer range, resulting in potentially unreliable results. However, accuracy of mass flow controllers allowed the desired accuracy for 0.1% CO_2 gas phase concentration. For all other CO_2 gas phase concentrations, the Nova gas analyzer model WP-375 was used to measure and display the CO_2 concentration of the gas stream.

I. Erlenmeyer Flask
When the three (3) liter flask was heated to the desired experimental temperature, the CO_2-air mixture was bubbled into the media flask at an approximate flow rate of 18.7 LPM. Omega mass flow controllers were used to maintain the CO_2 and compressed air flow rates to achieve the specific CO_2 concentration. The Nova gas analyzer model WP-375 was also used to measure the gas concentration by connecting its inlet with the exhaust of the experimental apparatus. The output of the gas analyzer was wired to the data acquisition system for automatic recording of the CO_2 level in the gas stream.

II. Falling Films
Approximately 71-L of media was heated in the growth tank and continuously pumped to three headers (one for each membrane substrate) at a total flow rate of 2.4 GPM per a linear foot of vertical membrane surface. The headers generated falling liquid films on both sides of each 2’x1’ membrane. When the medium temperature in the growth tank reached the experimental temperature, the CO_2- air mixture was introduced into the bioreactor, heated using two fin-strip heaters. The mixture was introduced to flow parallel with the membrane substrates. The CO_2 concentration in the gas stream was measured using the Nova gas analyzer and data acquisition system.

D. Temperature Measurement and Control

Solubility of CO_2 is temperature-dependent; therefore, temperature was controlled and maintained to ±1 ˚C for each experiment. Watlow series 93-temperature controllers, Type K thermocouples, and fin-strips and submersible heaters were used to achieve the target temperature for both setups.

I. Erlenmeyer Flask
Three Type K thermocouples were used for media temperature measurement and control: two (one for the media and the other one for the bath) for measurement and automatic record using the data acquisition system, and one for the controller. A submersible heater was used to achieve the desired temperature.

II. Falling Films
Four type K thermocouples were employed for temperature measurement and control: two for the media and the other two for the CO_2-enriched air stream. A submersible Tempco 750-Watt heater was installed on the growth tank wall to heat and control the medium temperature using a Watlow series 93-temperature controller. In addition, two fin-strip heaters were installed in the circulation air duct to heat and maintain the CO_2-enriched air stream using the Watlow series 93-temperature controller.

E. Measuring TIC Concentration over Time
An OI Analytical Model 1010 Wet Oxidation TOC analyzer was used in TIC mode to measure inorganic carbon concentration in samples of media. The TOC analyzer was calibrated every three weeks using four calibration standards and a blank to cover the expected TIC range. The correlation from each calibration must be at least 0.9990 to proceed. The manufacturer reported a detection range of 2 ppbC to 125 ppmC was possible without sample pretreatment, prepurging, or dilution [9]. However, our internal testing found that for a 99%

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confidence level determined from analysis of 15 replicates of a 0.1 mg/L (100 ppbC) standard, the reported level was 0.094 mg/L (94 ppbC). The TIC analyzer took approximately six minutes to complete each analysis and was set to an autosampler mode for continuously consecutive sampling until the TIC concentration reached steady state. For each experiment the CO_2-air mixture was introduced to the media immediately after the TOC analyzer was activated.

III. RESULTS

TIC measurements from the Erlenmeyer flask and falling film setups produced similar saturation TIC concentrations for all temperatures, CO_2-air concentrations and media. Therefore, only the results from the Erlenmeyer flask experimental are presented.

A. TIC Concentration for Each Experimental Temperature over Time

TIC concentrations were determined from averaged values of three runs of each sample taken and plotted with respect to time. In the first presentation of results for TIC concentrations at 25 ˚C, the trends observed between RO water and GM, and between RO+ and GM+ were very similar. Therefore, plots of TIC concentration over time for GM and GM+ are illustrated as in Figures 5 and 6.

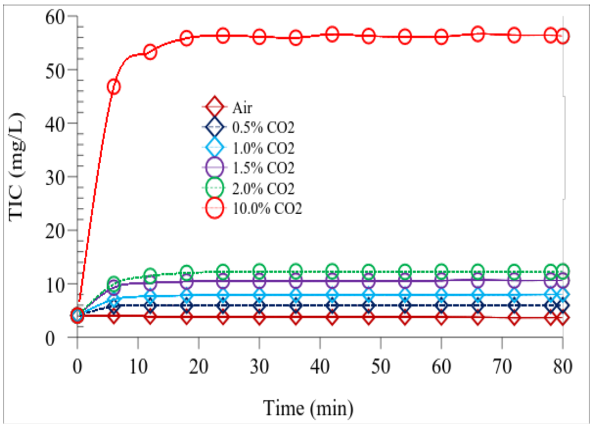

Fig. 5. TIC concentration in GM at 25˚C as a function of time

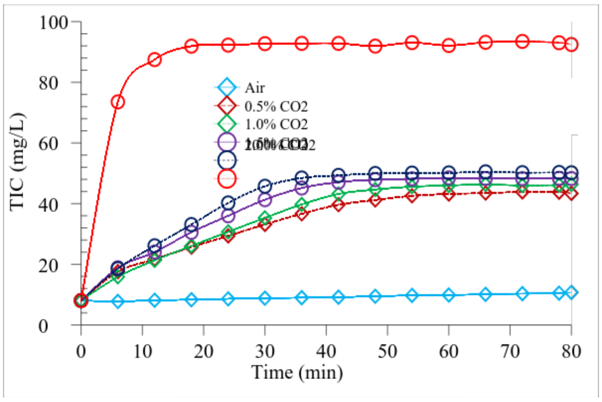

Fig. 6. TIC concentration in GM+ at 25˚C as a function of time

Compared to Figure 5, the data in Figure 6 show that when adding the NaOH solution into the media, the time required to reach saturation was longer and the saturated (maximum) TIC concentration was higher that without the NaOH solution.

B. Changing of Media pH over Time

Media pH from the data acquisition was plotted with respect to time during TIC measurements to examine trends of pH and TIC concentration with respect to time. Figures 7 and 8 illustrate changes of media pH in GM and GM+ at 25˚C for gas phase concentrations of CO_2 of air, 0.5%, 1.0%, 1.5%, 2.0%, and 10.0% respectively.

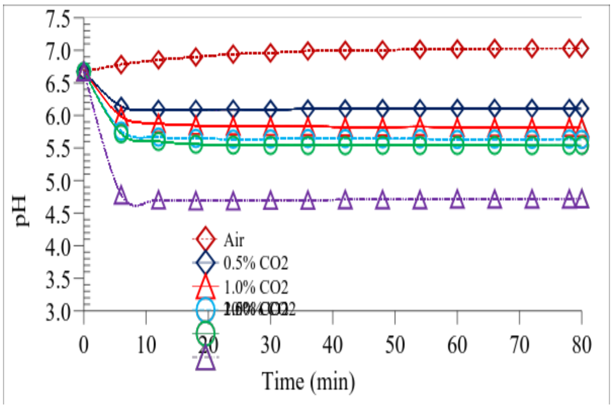

Fig. 7. GM pH at 25˚C as a function of time

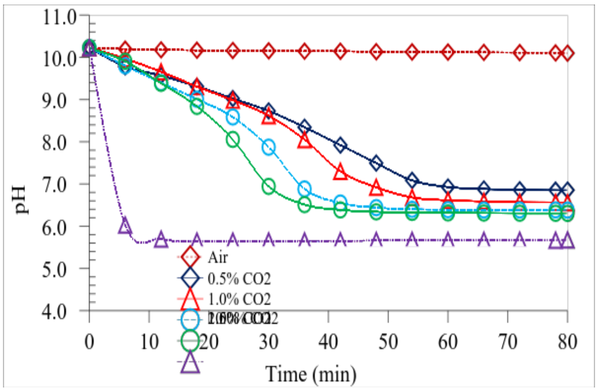

Fig. 8. GM+ pH at 25˚C as a function of time

In addition, adding the NaOH solution into RO water and GM increased the saturated pH approximately 1 unit pH compared to without NaOH for a specific CO_2 level and temperature. Also, media pH became steady at the same time as seen measuring TIC levels. This indicates that pH is a possible indicator of TIC, especially at saturation.

C. Determining Saturated TIC Concentration

The average TIC concentration from three replicates for each medium, temperature and CO_2 levels was used to calculate the saturated TIC concentration. Saturated TIC concentration for this study was defined as the TIC concentration mean of at least five consecutive data points with less than 1% difference. Percentage difference was calculated as the difference between the one TIC concentration measurement and a consecutive value,

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dividing by the last value, and then multiplying by 100. Each experimental condition, except ambient air, must consist of at least five consecutive data points providing less than 1.0% difference. Figure 9 illustrated the determination of 1.0% difference for 0.5% and 1.0% CO_2.

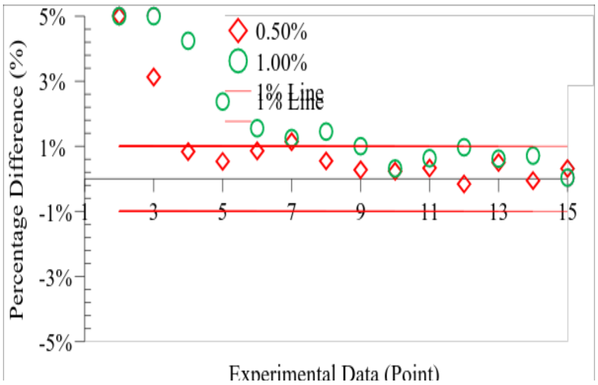

Fig. 9. Determining the saturated TIC concentration for absorption of CO_2 concentrations of 0.5% and 1.0% in GM at 25˚C

Table 2 Experimental results for saturated TIC concentration

CO_2 (%)	T (˚C)	Saturated TIC (mg/L)			
		RO	RO+	GM	GM+
Air	25	0.261	9.093	3.693	10.747
	35	0.177	10.617	3.698	11.051
	45	0.071*	9.350	3.583	12.807
	55	0.048*	13.333	3.579	13.251
0.1	25				40.710
	35				40.870
	45				40.982
	55				40.710
0.5	25	2.909	41.386	5.954	43.630
	35	2.189	40.789	5.548	42.740
	45	1.678	40.783	5.147	43.117
	55	1.285	41.884	4.764	44.396
1.0	25	5.340	43.711	7.978	46.149
	35	4.142	41.655	7.354	43.779
	45	3.209	42.236	6.550	44.067
	55	2.514	41.673	6.134	43.403
1.5	25	7.974	45.965	10.589	48.329
	35	6.330	43.277	9.396	46.636
	45	5.120	43.529	7.796	45.841
	55	4.478	42.026	7.367	44.974
2.0	25	9.673	48.070	12.264	50.230
	35	7.510	46.114	11.010	49.606
	45	6.430	44.829	9.047	48.043
	55	5.535	43.422	8.670	45.754
10.0	25	52.062	89.342	56.306	92.769
	35	39.265	78.069	42.603	81.233
	45	31.976	70.567	34.550	74.233
	55	25.988	64.216	29.125	67.920

Once the criterion for saturation was reached, at least five consecutive TIC concentrations at this condition were then averaged and that value was considered the saturated TIC concentration. In addition, these data were also used to calculate standard deviation (STDEV), coefficient of variance (CV), absolute and relative errors, which were very small. Saturated TIC values from each medium are shown in Table 2.

* below the minimally reliable threshold detection limit of 94 ppb

D. Saturated TIC Concentration as a Function of Temperature

Saturated TIC concentration for a specific gas phase CO_2 concentration decreased with respect to increasing temperature with the greatest effect at low temperature, as shown in Figure 10. However, the addition of NaOH solution increased the inorganic carbon holding capacity as shown in Figure 11. Thus, the effect of temperature on TIC concentration among 0.1%, 0.5%, 1.0%, 1.5% and 2.0% CO_2 was insignificant in the range of 25-55°C when NaOH was added to the media.

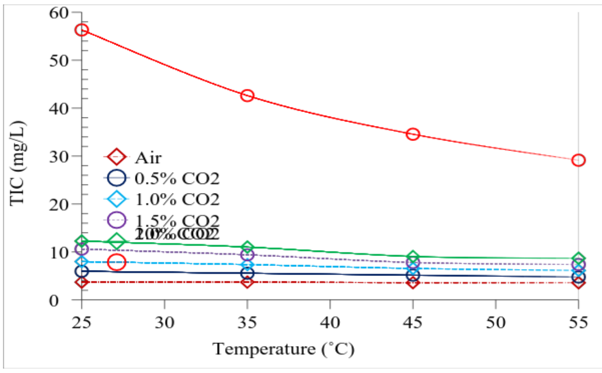

Fig. 10. Saturated TIC concentrations in GM as a function of temperature

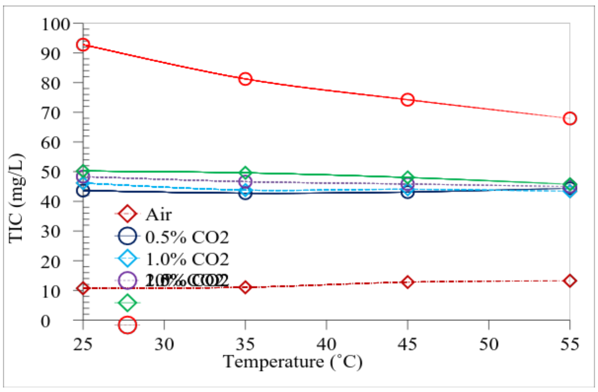

Fig. 11. Saturated TIC concentrations in GM+ as a function of temperature

E. Comparison of Saturated TIC Concentration

Excluding ambient air, the saturated trend of TIC concentration with respect to temperature between RO water and GM, and RO+ and GM+ were very consistent. The average TIC concentration difference and the standard deviation were 3.127 mg/L and 0.438 for RO water and

GM, and 2.665 mg/L and 0.640 for RO+ and GM+, respectively. It is clear that the difference in TIC concentration between RO and GM was from inorganic chemical species existing in GM that reacted and formed additional inorganic carbon rather from absorption of CO_2. When mixing RO water and GM with the NaOH solution, the saturated TIC differences between RO and RO+, and GM and GM+ were 38.397 and 37.935 mg/L, respectively. It is clear that adding the NaOH solution into RO water and GM improved the CO_2 solubility. Adding the NaOH solution into RO water can form two additional chemical inorganic carbon species: $NaHCO_3$ and $NaCO_3^-$ already in GM, which led to a decrease of the saturated TIC difference between RO+ and GM+ compared to RO and GM. The saturated TIC concentrations shown in Table 2 were used to examine the saturated TIC difference between media with and without the NaOH solution as illustrated in Table 3.

Table 3 Experimental saturated TIC difference

CO_2 (%)	T (˚C)	TIC difference (mg/L)			
		RO-RO+	RO-GM	GM-GM+	RO+-GM+
Air	25	8.832	3.432	7.054	1.654
	35	10.44	3.521	7.353	0.434
	45	9.279	3.512	9.224	3.457
	55	13.285	3.531	9.672	0.082
0.5	25	38.477	3.045	37.676	2.244
	35	38.600	3.359	37.192	1.951
	45	39.105	3.469	37.970	2.334
	55	40.599	3.479	39.632	2.512
1.0	25	38.371	2.638	38.171	2.438
	35	37.513	3.212	36.425	2.124
	45	39.027	3.341	37.517	1.831
	55	39.159	3.620	37.269	1.730
1.5	25	37.991	2.615	37.740	2.364
	35	36.947	3.066	37.240	3.359
	45	38.409	2.676	38.045	2.312
	55	37.548	2.889	37.607	2.948
2.0	25	38.397	2.591	37.966	2.160
	35	38.604	3.500	38.596	3.492
	45	38.399	2.617	38.996	3.214
	55	37.887	3.135	37.084	2.332
10.0	25	37.280	4.244	36.463	3.427
	35	38.804	3.338	38.630	3.164
	45	38.591	2.574	39.683	3.666
	55	38.228	3.137	38.795	3.704
Average		38.397	3.127	37.935	2.665
STDEV		0.792	0.438	0.913	0.640

F. Possible Formulae for TIC Concentration from Absorbed CO_2

RO water does not contain chemicals that can form inorganic carbon without the absorption of CO_2. Therefore, the TIC concentration in RO water was only from dissolved CO_2. In addition, inorganic carbon concentration influenced media pH as shown in Figures 7 and 8 and then became available carbon species for aquatic photosynthetic organisms.

GM contains various chemicals that can later form chemical inorganic carbon additionally from the CO_2 solubility. This additional inorganic carbon is the TIC difference between RO water and GM, and RO+ and GM+. Therefore, formulae for the TIC concentrations in RO water, RO+, GM and GM+ can be written as shown in Equations (5) and (6), respectively.

RO water $$TIC = IC_{CO_2} \quad (5)$$

RO+, GM and GM+ $$TIC = IC_{CO_2} + IC_P \quad (6)$$

Where, IC_{H_2O} is a sum of aqueous CO_2, carbonic acid, bicarbonate, and carbonate from the CO_2 solubility and IC_P is a sum of inorganic carbons forming from existing chemicals in the media. The chemical reactions forming chemical inorganic carbons may vary with temperature and CO_2-enriched air, but they were consistent in this study.

G. Influence of NaOH Concentration on CO_2 Absorption

When adding the NaOH solution into the media, the time required to achieve saturated TIC concentration was longer and the saturated TIC concentration was higher as already illustrated in Figure 6. This circumstance can imply that the NaOH increased the ability of the media to absorb more TIC from the CO_2 solubility.

Different concentrations of NaOH solution were also used to mix with GM to investigate impact of the NaOH solution concentration on CO_2 absorption and TIC formation. The study was conducted using CO_2 gas phase concentrations of 0.5% and 1.0% at temperatures of 25 and 45 ˚C. The concentrations of 0.5, 1.5 and 3.0 mM NaOH solution with GM were studied with 1.0% CO_2, whereas 1.5 and 3.0 mM NaOH was used with 0.5% CO_2. The experimental results are provided in Table 4.

Table 4 Saturated TIC in GM vs NaOH concentrations

% CO_2	T (˚C)	NaOH (mM)	Initial pH	Saturated TIC concentration (mg/L)
1.0	25	0	6.7±0.2	7.798
		0.5	9.1±0.2	14.923
		1.5	9.8±0.2	26.509
		3.0	10.5±0.2	43.168
	45	0	6.7±0.2	6.550
		0.5	9.1±0.2	12.961
		1.5	9.8±0.2	24.514
		3.0	10.5±0.2	41.855
0.5	25	0	6.7±0.2	5.594
		1.5	9.8±0.2	24.032
		3.0	10.5±0.2	43.630

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	45	0	6.7±0.2	5.147
		1.5	9.8±0.2	23.125
		3.0	10.5±0.2	43.711

The saturated TIC data for the CO_2 concentrations of 0.5% and 1.0% at 25 and 45˚C from experiments increased with respect to the concentration of the NaOH solution. Figure 12 shows the saturated TIC concentration for CO_2-enriched air of 1.0% with respect to the concentration of the NaOH solution. Data from Figure 12 indicates that the saturated TIC concentration linearly increased with respect to the NaOH concentration. For each specific NaOH concentration, the distance between two experimental temperatures is the saturated TIC concentration difference for these two temperatures.

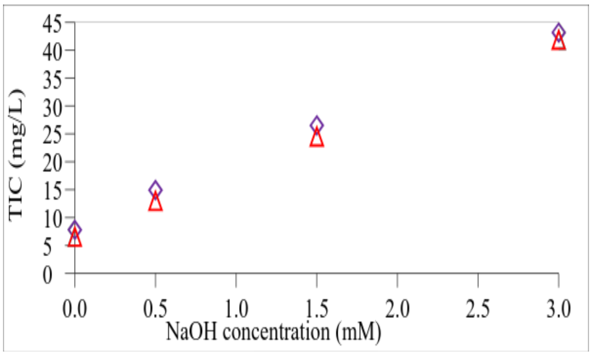

Fig 12. Saturated TIC concentration for 1.0% CO_2 in GM vs NaOH solution concentrations (and for experiment at 25 and 45˚C)

IV. CONCLUSION

The time required to achieve saturated TIC from the absorption of CO_2 in experimental media for each condition was inversely proportional to the concentration of gas phase CO_2. Sodium hydroxide solution added to the media raised the TIC holding capacity, which resulted in a longer time to reach saturation especially in the case of ambient air and 0.1% CO_2 due to low CO_2 mass transfer rates.

The TIC holding capacity of the media increased proportionally to the amount of NaOH solution. However, based on the criteria for the saturated TIC, excluding air and 0.1% CO_2, 15 TIC measurements for each experimental condition provided sufficient data to find the TIC saturation concentration. The saturated TIC data for each medium and concentration of CO_2 decreased with respect to increased temperature, but fluctuated with temperature for CO_2 concentrations of 0.5% and 1.0% in media of RO+ and GM+. Also, the TIC data indicate that RO water and cyanobacteria growth media have a similar behavior of the CO_2 solubility. However, chemical species contained in the growth media may form additional inorganic carbon as described in Equations (3.5) and (3.6).

The experimentally saturated TIC values from the CO_2 absorption in RO water and the growth media were proportional to the partial pressure of CO_2. However, when the TIC holding capacity was increased using the NaOH solution, the saturated TIC values in RO+ and GM+ were disproportional to concentrations of CO_2-enriched air.

Media pH was inversely proportional to TIC concentration and the CO_2 partial pressure for all temperature, media and CO_2 concentrations. Data also demonstrated that increasing TIC during CO_2 mass transfer decreased the medium pH rapidly and the media pH became stabilized as the TIC reached saturation. In addition, chemical species in GM and the NaOH solution in RO+ and GM+ increased the saturated pH of the media. As the saturated TIC values decreased with respect to increased temperature, the saturated medium pH increased with respect to increased temperature.

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BIOGRAPHY

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