CHAPTER III
MATERIALS AND METHODS

Experimental methods can be divided into two parts; (1) maximising CO_2 dissolution in bubble column, and (2) the usage of bicarbonate as inorganic carbon source for fresh water microalgae *Chlorella vulgaris*. The first and second parts are illustrated in Figures 3.1 and 3.2, respectively. For CO_2 dissolution study, many manipulated variables are examined and then finally evaluated for the optimal dissolution condition. This can be elucidated using Figure 3.1. Figure 3.2 displays the details of experiments in which bicarbonate is employed as inorganic carbon source for fresh water microalgae cultivation. *C. vulgaris* is chosen to be the fresh water algal model in this work due to its rapid growth rate and ease of system maintenance.

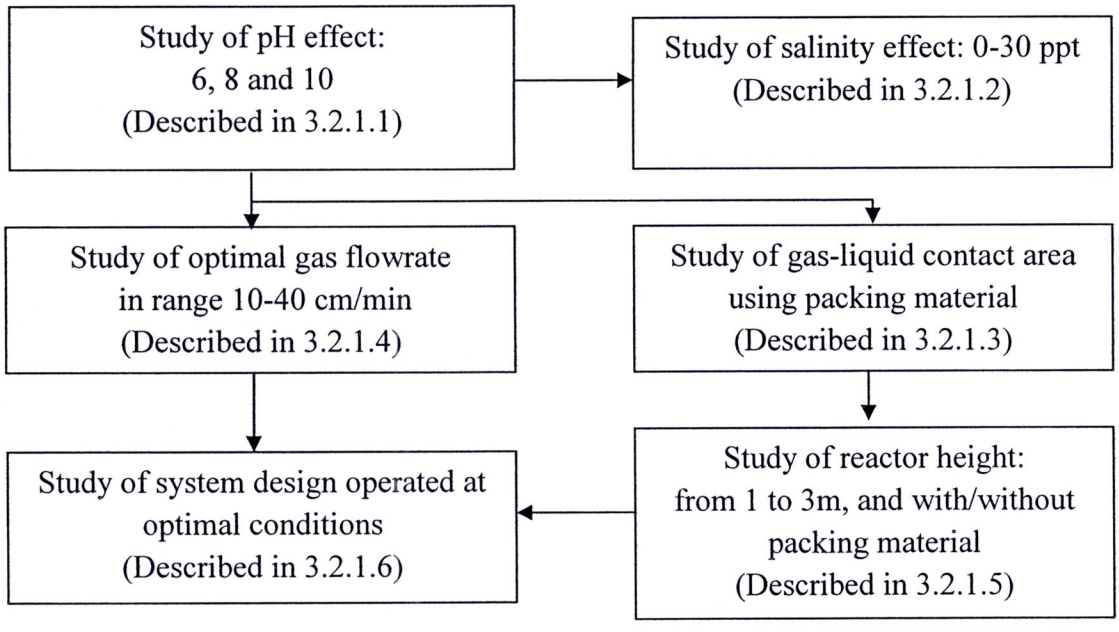

Figure 3.1 Experimental diagram: Maximising CO_2 dissolution in bubble column

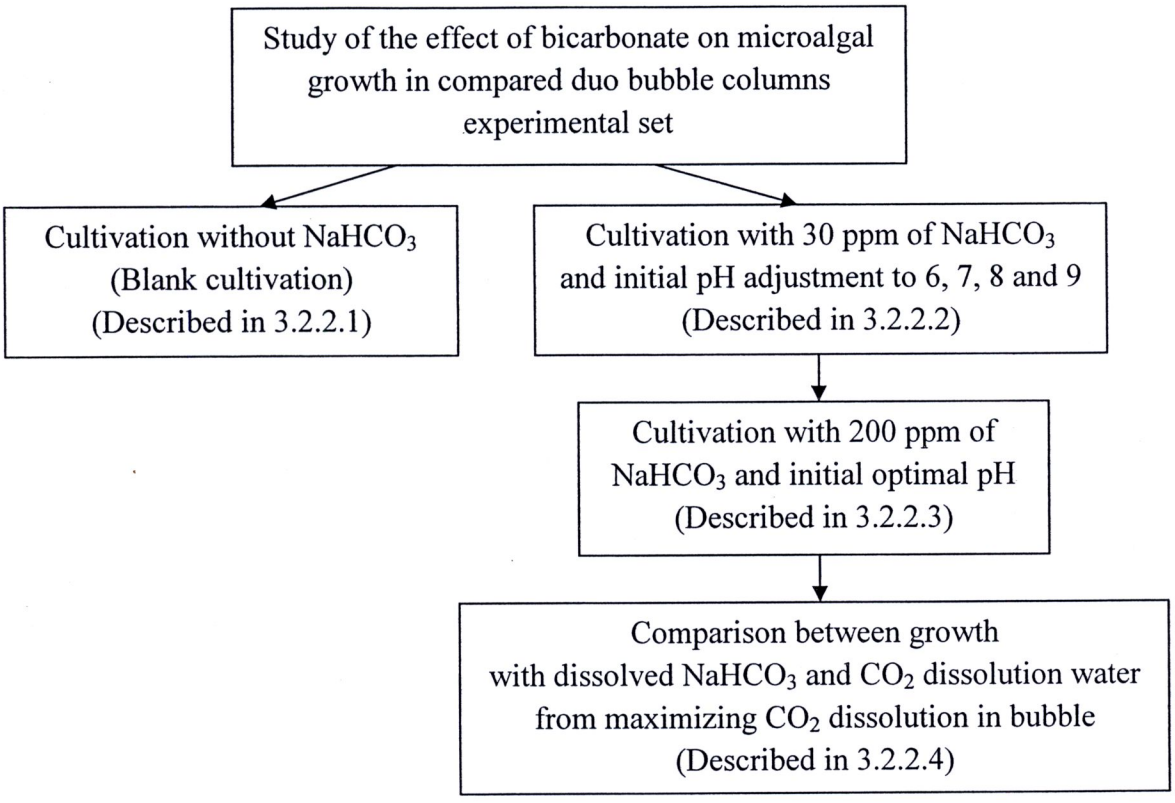

Figure 3.2 Experimental diagram: Study of bicarbonate as inorganic carbon source for fresh water microalgae cultivation

3.1 Experimental Setup

3.1.1 Combined effect of system design for CO_2 dissolution

3.1.1.1 Experiments in bubble columns

The effect of pH and salinity on CO_2 dissolution in the water is investigated in a 1 m high clear acrylic plastic bubble column with a diameter of 6.3 cm (see Fig. 3.3 for a schematic of the bubble column). In case of study of optimal gas flowrate, 2 metre high bubble column with the same diameter is instead employed. CO_2 gas is fed from gas cylinder and measured with a "Dawyer" rotameter before entering the reactor at the bottom. The gas velocity is controlled and sparged at 10 cc min^{-1}.

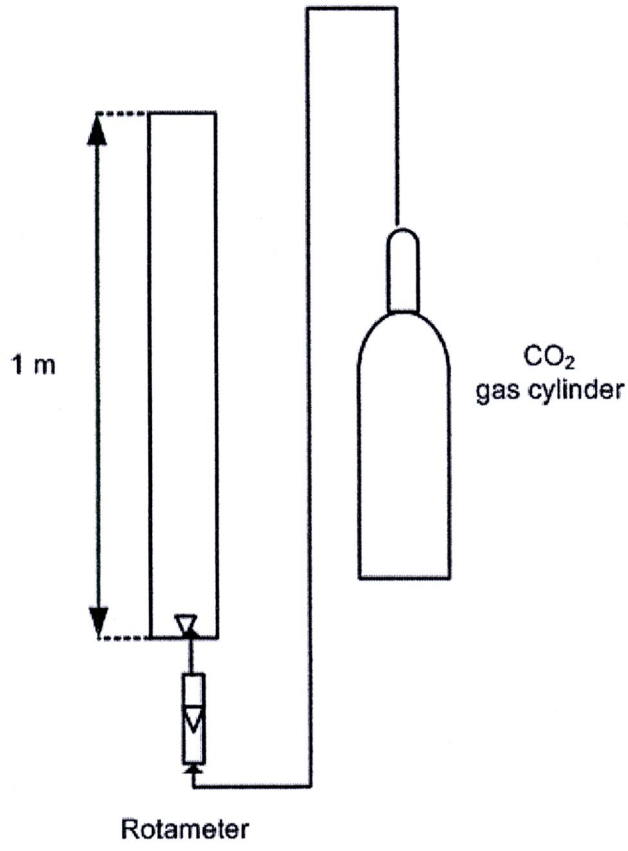

Figure 3.3 Experimental setup for CO_2 fed bubble column

3.1.1.2 Experiments in packed columns

The effect of gas-liquid contact area on CO_2 dissolution is studied in a 1 m high clear acrylic plastic bubble column. This is exactly the same column as that used in Section 3.1.1.1 but this time fully filled with 1 cm diameter packing material. In case of study effect of height in packed material, 3 metre high packed column with equal diameter is replaced. CO_2 gas is fed from gas cylinder and measured with a "Dawyer" rotameter before entering the reactor at the bottom. The gas velocity is controlled and sparged at 10 cc min^{-1}.

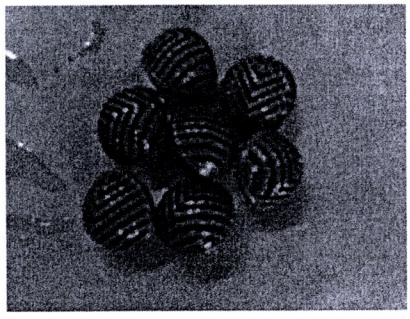

Figure 3.4 One cm diameter packing material

3.1.1.3 Circulating Counterflow Contactor (C.C.C.) packed column

The combined effect of system design for optimal CO_2 dissolution is investigate in "C.C.C" in which 1 m high packed column is fully filled with packing material. Water is allowed to circulate in transverse direction with gas fed (see Fig.3.4). Water recycle flowrate is manipulated with rotameter at a desire range of 1-3 LPM (Litres per minute). Submerged pump is placed in 5 Litres reservoir enabling water to circulate further. CO_2 gas is fed from gas cylinder and measured with a "Dawyer" rotameter before entering the reactor at the bottom. The gas velocity is controlled and sparged at 10 cc min^{-1}.

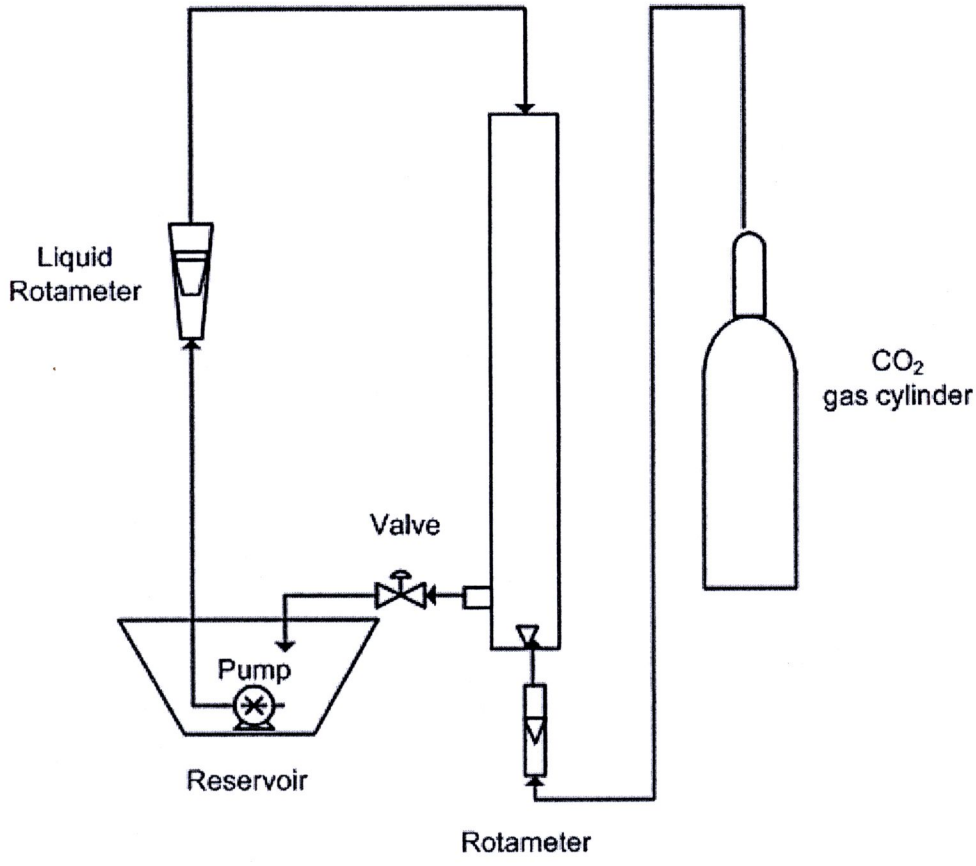

Figure 3.5 Experimental setup for Circulating Counterflow Contactor

3.1.2 Effect of bicarbonate as inorganic carbon source for fresh water microalgae

Two 2.8 L bubble columns are prepared side by side to allow the sharing of light source as illustrated in Fig 3.4. This system is thereafter called "duo bubble columns". 18 W Fluorescent light bulbs are located between the two columns illuminating 10000 Lux light intensity. Fresh water is sterilised by 50 ppm (parts per million) of chlorine (as sodium hypochloride), air is measured with "Dawyer" rotameter and supplied at a superficial velocity (U_{sg}) = 1 cm/s.

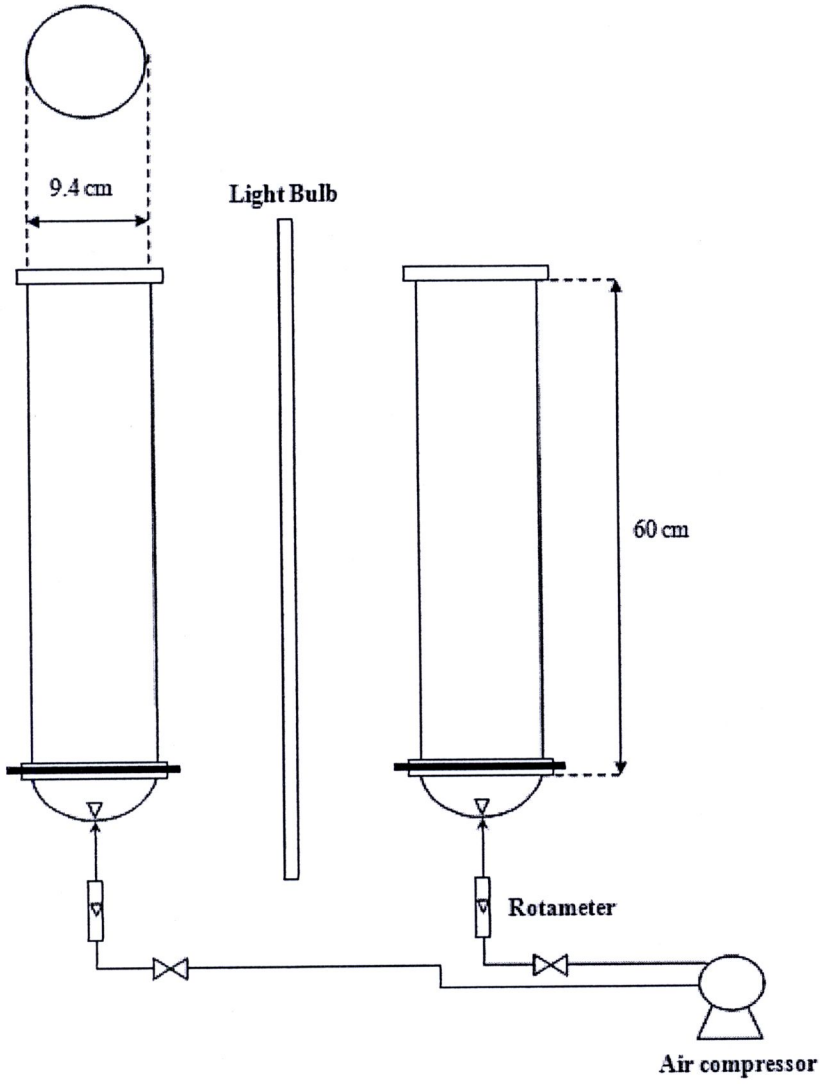

Figure 3.6 Experimental setup for cultivation of *Chlorella vulgaris* in sharing light source bubble columns

3.2 Experimental Procedure

3.2.1 Maximising CO_2 dissolution in bubble column

3.2.1.1 Study of pH effect

1. Setup the sparged bubble column as described in Section 3.1.1.1
2. Adjust demineralised water with HCl 0.5 M and NaOH 0.5 M at pH of 6, 8 and 10.
3. Sparge CO_2 from the gas cylinder to the porous sparger at the bottom of the column.
4. Collect 20 ml sample at every 15 minutes for one hour; store the collected samples in refrigerator.
5. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.1.2 Study of salinity effect

1. Setup the sparged bubble column as described in Section 3.1.1.1
2. Replace fresh water with sea water at salinity of 5, 10 and 30 ppt
3. Sparge CO_2 from the gas cylinder to the porous sparger at the bottom of the column.
4. Collect 20 ml at every 15 minutes for one hour; store the collected samples in refrigerator.
5. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.1.3 Study of gas-liquid contact area effect

1. Setup the sparged packed column as described in Section 3.1.1.2
2. Adjust demineralised water with HCl 0.5 M and NaOH 0.5 M at pH of 6, 8 and 10.
3. Sparge CO_2 from the gas cylinder to the porous sparger at the bottom of the column.
4. Collect 20 ml at every 15 minutes for one hour; store the collected samples in refrigerator.
5. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.1.4 Study of optimal gas flowrate effect

1. Setup the sparged packed column as described in Section 3.1.1.2
2. Adjust demineralised water at optimal pH obtained from Section 3.2.1.1 with HCl 0.5 M and NaOH 0.5 M
3. Sparge CO_2 at 10, 20, 30 and 40 from the gas cylinder to the porous sparger at the bottom of the column.
4. Collect 20 ml at every 15 minutes for one hour; store the collected samples in refrigerator.
5. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.1.5 Study of height and gas-liquid contact area effect

1. Setup the sparged packed column as described in Section 3.1.1.2
2. Adjust demineralised water with NaOH 0.5 M and HCl 0.5 M at optimal pH from obtained Section 3.2.1.1
3. Sparge CO_2 from the gas cylinder to the porous sparger at the bottom of the column.
4. Collect 20 ml at every 15 minutes for one hour; store the collected samples in refrigerator.
5. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.1.6 Study of combined effect of optimal conditions

This experiment employs the combined optimal conditions obtained from Sections 3.2.1.1-3.2.1.5 in order to determine the best condition for CO_2 dissolution in the bubble column.

1. Setup the sparged bubble column as described in Section 3.1.1.3
2. Adjust demineralised water with NaOH 0.5 M and HCl 0.5 M at optimal pH obtained from Section 3.2.1.1
3. Manipulate the recycle water flowrate at 1, 2 and 3 LPM
4. Sparge CO_2 from the gas cylinder to the porous sparger at the bottom of the column.

5. Collect 20 ml at every 15 minutes for one hour; store the collected samples in refrigerator.

6. Analyse for Total Inorganic Carbon (TIC) by using Shimazsu Total Inorganic Carbon (TOC) Analyser

3.2.2 Effect of bicarbonate on microalgal growth

Study of bicarbonate and pH effect on microalgal, *Chlorella vulgaris* growth can be divided into 4 experiments; 3.2.2.1 Blank cultivation (controlled experiment) –no bicarbonate added and no pH adjustment, 3.2.2.2 Cultivation with 30 ppm of $NaHCO_3$ and pH adjustment in range of 6-9, 3.2.2.3 Cultivation with 200 ppm of $NaHCO_3$ at optimal pH from 3.2.2.2, and finally 3.2.2.4 cultivation with the medium prepared from the Section 3.2.1.5 (with CO_2 dissolution). Next, 3.2.2.1-3.2.2.3 experiments are initially conducted by this followings.

1. Setup the bioreactors as described in Section 3.1.2

2. Sterilise the duo bubble columns and fresh water with 50 ppm chlorine (as sodium hypochloride). Sparge air through the porous sparger at the bottom of the column for about 1 day. Check the residual chlorine in fresh water by adding potassium iodide in fresh water, and if chlorine is not exhausted, the sample will be turned yellow.

3. Fill the column with sterilised culture medium together with pure culture with initial cell concentration of $1x10^6$ cells mL^{-1} and adjust the total working volume to 2.8 L

4. Supply the compressed air through a porous sparger and adjust the superficial gas velocity to 1 m s^{-1}

5. Supply both sides of column with 18 W fluorescent light bulbs, placed along the column height. The light intensity is adjusted in the range of 9,000 – 10,000 luxes or 122-136 μmol photon $m^{-2}s^{-1}$ by moving the light bulbs in or out from the column as shown in Figure 3.5.

6. Further experiment method for different conditions will be decribed in 3.2.2.1, 3.2.2.2 and 3.2.2.3.

3.2.2.1 Cultivation of Chlorella vulgaris without $NaHCO_3$ added and pH adjustment

1. Set up bioreactor as described in 3.2.2

2. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2).

3. Calculate the specific growth rate using Equation 3.3

3.2.2.2 Effect of bicarbonate and pH on microalgal growth

1. Setup bioreactor as described in 3.2.2

2. Setup 30 $mg \cdot L^{-1}$ bicarbonate concerntration in both reactors by adding dried $NaHCO_3$.

3. Adjust pH to 6, 7, 8 and 9 in both reactors by acid/base injection; NaOH 0.5 M and HCl 0.5 M

4. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2).

5. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4 and the specific productivity using Equation 3.5

3.2.2.3 Effect of 200 ppm of $NaHCO_3$ on microalgal growth

1. Setup bioreactor as described in 3.2.2

2. Setup 200 $mg \cdot L^{-1}$ bicarbonate concentration in both reactors by adding dried $NaHCO_3$

3. Adjust pH to optimal pH (obtained from 3.2.2.2) in both reactors by acid/base injection; NaOH 0.5 M and HCl 0.5 M

4. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2).

5. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4, and the specific productivity using Equation 3.5

3.2.2.4 Effect of CO_2 dissolution water from optimal combined effect bubble column

1. Setup the bioreactors as described in Section 3.1.2

2. Sterilize the duo bubble columns with 50 ppm chlorine (as sodium hypochloride). Sparge air through the porous sparger at the bottom of the column for about 1 day. Check the residual chlorine in fresh water by adding potassium iodide in fresh water, and if chlorine is not exhausted, the sample will be turned yellow.

3. Release the sterilized water from the bioreactor.

4. Fill the column with CO_2 dissolution water from 3.2.1.5

5. Fill the column with sterilized culture medium together with the pure culture with initial cell concentration of $1x10^6$ cells mL^{-1} and adjust the total working volume to 2.8 L

6. Supply the compressed air through a porous sparger and adjust the superficial gas velocity to 1 m s^{-1}

7. Supply both sides of column with 18 W fluorescent light bulbs, placed along the column height. The light intensity is adjusted in the range of 9,000 – 10,000 luxes or 122-136 μmol photon $m^{-2}s^{-1}$ by moving the light bulbs in or out from the column as shown in Figure 3.5.

8. Adjust pH to optimal pH from 3.2.2.2 experiment in both reactors by acid/base injection; NaOH 0.5 M and HCl 0.5 M

9. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2).

10. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4 and the specific productivity using Equation 3.5

3.3 Analyses

3.3.1 Determination of Total Inorganic Carbon (TIC)

Dissolved inorganic carbon (DIC) is the sum of inorganic carbon species in a solution. The inorganic carbon species include carbon dioxide, carbonic acid, bicarbonate anion, and carbonate. It can express carbon dioxide and carbonic acid simultaneously as

CO_2*. C_T is a key parameter when making measurements related to the pH of natural aqueous systems, and carbon dioxide flux estimates.

$$C_T = [CO_2^*] + [HCO_3^-] + [CO_3^{2-}]$$

where,

C_T is the total inorganic carbon concentration ($mg \cdot L^{-1}$)

$[CO_2^*]$ is the sum of carbon dioxide and carbonic acid concentrations ($[CO_2^*] = [CO_2] + [H_2CO_3]$) ($mg \cdot L^{-1}$)

$[HCO_3^-]$ is the bicarbonate concentration ($mg \cdot L^{-1}$)

$[CO_3^{2-}]$ is the carbonate concentration ($mg \cdot L^{-1}$)

Total inorganic carbon is determined by Acidification of phosphoric acid operating in removal and venting of IC and POC (Particulate Organic Carbon) gases from the liquid. Then, IC mostly transforms into CO_2 and it is detected by the non-dispersive infrared analysis (NDIR). A region of adsorption of infrared light specific to CO_2, usually around 4.26 μm (2350 cm^{-1}), is measured over time as the gas flows through the detector. The gas continues to flow into and out of the detector cell, the sum of the measurements results in a peak that is integrated and correlated to the total CO_2 concentration or Total Inorganic carbon totally found in the sample, in concentration, mg/l.

3.3.2 Determination of %CO_2 dissolution efficiency

%Efficiency of CO_2 dissolution for each time can be determined from proportion of CO_2 dissolved as TIC in mg divided by the total amount of CO_2 entering the reactor in mg:

$$\%Efficiency = \frac{TIC \times V_L}{\left(\frac{PQ_g tM_w}{RT}\right)} \tag{3.1}$$

where

TIC = Total Inorganic Carbon concentration ($g \cdot L^{-1}$)

V_L = Volume of water in bubble or packed column (L)

P = Pressure (bar)

Qg = Volumetric flowrate of CO_2 fed in the system ($L \cdot min^{-1}$)

t = Time operated (min)

Mw = Molecular weight ($g \cdot mol^{-1}$)

R = Gas constant = 82 $bar \cdot L \cdot mol^{-1} \cdot K^{-1}$

T = Temperature (K)

3.3.3 Determination of cell concentration

Due to the very high density of *Chlorella sp.*, appropriate dilution is needed. Cell concentration is determined using Haemacytometer. The depth of the counting grid and the medium area are 0.1 mm and 0.04 mm^2, respectively. The cell concentration can be determined as follows:

1. Clean the counting slide and cover glass
2. Fill the slide with sample
3. Cover the slide with cover glass, avoid the presence of bubbles
4. Count the cell in 25 medium squares on the grid

5. Calculate the cells number, using Equation 3.2:

$$N = \frac{n}{25} \times 10^5 \tag{3.2}$$

where

N = cells concentration (cells·mL^{-1})

n = number of cells on 25 squares in upper and lower grid (cells)

3.3.4 Determination of specific growth rate

The specific growth rate can be calculated from Equation 3.3 as follows:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \tag{3.3}$$

where

μ = specific growth rate (h^{-1})

N_1 = cells concentration at t_1 (cells·mL^{-1})

N_2 = cells concentration at t_2 (cells·mL^{-1})

t_1 = first sampling time (h)

t_2 = second sampling time (h)