

# **CO<sub>2</sub> Sequestration by *Chlorella vulgaris***

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In  
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## CERTIFICATE

This is to certify that the dissertation entitled “**CO<sub>2</sub> Sequestration by *Chlorella vulgaris***”, is an authentic record of my own work carried out as requirements for the award of degree of Master of Technology in Chemical Engineering from Thapar University, Patiala, under the guidance of Dr. Pramod K. Bajpai (Distinguished Professor, ChED) and Dr. Haripada Bhunia (Associate Professor, ChED) from July 2012 to June 2013.

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**KUMAR UDDIPTO**

## ABSTRACT

Rising carbon dioxide levels in the environment is a cause for serious concern. The rising levels of carbon dioxide can be dealt with in many ways. One of the ways is to reduce energy usage by optimizing process efficiency or by using renewable fuel sources. Another way is to capture the CO<sub>2</sub> at the source of its emission. What sets apart, bio-sequestration from other carbon capture and storage (CCS) methods, is that it can help in reduction of CO<sub>2</sub> using both of the strategies mentioned earlier. Micro-algal capture of CO<sub>2</sub> using photo-bioreactors (PBR) allows greater photosynthetic efficiency over traditional plant growth. Both these factors combined, lead to better CO<sub>2</sub> uptake or carbon dioxide assimilation. Optimal conditions of growth along with proper selection of culture(s) may allow additional benefits – bio-fuel production, protein production, etc.

*Chlorella* strains have already been reported to have good CO<sub>2</sub> sequestration capability as well as potential to produce biodiesel, which was why it was selected for our studies. All studies were carried out at 10% inlet CO<sub>2</sub> in shake flasks containing 200 mL media with 10% inoculum by volume.

Maximum specific growth rate and maximum carbon fixation rates were 0.596 mg L<sup>-1</sup> day<sup>-1</sup> and 586.5 mg L<sup>-1</sup> day<sup>-1</sup>, respectively.

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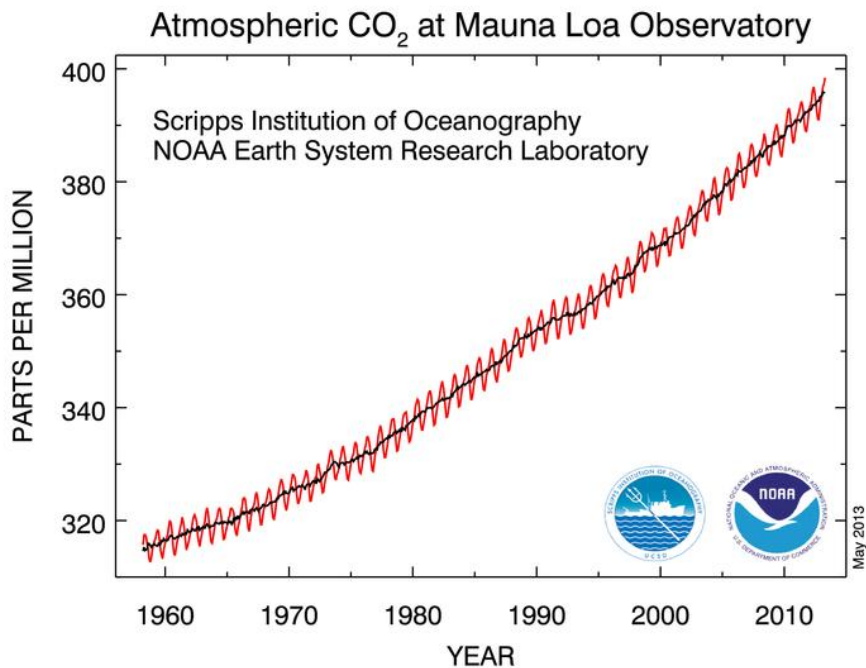
## CHAPTER- 1 INTRODUCTION

Global warming is a serious environmental problem. This is generally attributed to an increase in the atmospheric concentration of greenhouse gases (GHGs), particularly carbon dioxide (CO<sub>2</sub>), methane, tropospheric ozone, and chlorofluorocarbons [1]. However, it is CO<sub>2</sub> which contributes the most to global warming out of all GHGs.

Natural sources of carbon dioxide are almost 20 times greater than that due to human interference(s). However, over the years, it is closely balanced out by natural sinks such as forests, water bodies, marine flora etc. Therefore, for a long time upto mid 19<sup>th</sup> century, the net carbon dioxide levels in the atmosphere remained the same, approximately 260 – 280 ppm [2]. Human interference in these natural cycles due to industrial advancement has led to an increase in carbon dioxide levels. The average temperature of earth's atmosphere has been steadily increasing since the last 100 years. Although, there is no scientific evidence which establishes this beyond doubt, the fact remains that surface temperatures of the earth have risen steadily over the last 100 years by at least 1 °C. Scientists add that there is a 40 % probability of surface temperatures continuing to rise and only a 5% chance of it decreasing, over the next 50 years. Solid land masses are more sensitive to temperature changes than water bodies. Hence, the temperatures of continent landmasses will rise faster than oceans [3]. According to the 4<sup>th</sup> IPCC Assessment Report, atmospheric concentration of CO<sub>2</sub> was 379 ppm, far beyond the natural range of last 650,000 years (180-300 ppm) [4] which has been increasing with time as shown in Figure 1.1. The world recognizes the potential adverse effects of global climate change and has adopted the United Nations Framework Convention on Climate Change (UNFCCC), whose objective is stated as - stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system [5].

Three different approaches may be followed in order to reduce the rising CO<sub>2</sub> concentrations-

1. Increase efficiency in electricity generation and its optimum use
2. Expand use of renewable energy sources such as wind, solar, biomass, geothermal and other alternate energy sources, viz. nuclear power
3. Capture carbon dioxide emissions at fossil-fueled (especially coal) electric generating plants and permanently sequester the carbon.



**Figure 1.1** Atmospheric CO<sub>2</sub> variations over the years

It is worthy to mention that we may need to adopt all of these available options and hence, considering the present scenario, it would be a mistake excluding any of these options from an overall carbon emissions management strategy. Sequestrations of CO<sub>2</sub> from the industries are today's demand in order to reduce the impact of CO<sub>2</sub> on global warming. Carbon dioxide sequestration can be defined as the secure storage of CO<sub>2</sub> that would otherwise be emitted to or remain in the atmosphere. The idea is to keep carbon emissions produced by human activities from reaching the atmosphere by capturing and diverting them to secure storage or to remove carbon from the atmosphere by various means and store it. All physico-chemical processes for CO<sub>2</sub> fixation involve three stages (Figure 1.2) –

1. Capture of CO<sub>2</sub>.
2. Transportation of this captured CO<sub>2</sub>
3. Long term storage of this CO<sub>2</sub>, in stable inorganic form so that it doesn't revert back to its original state.

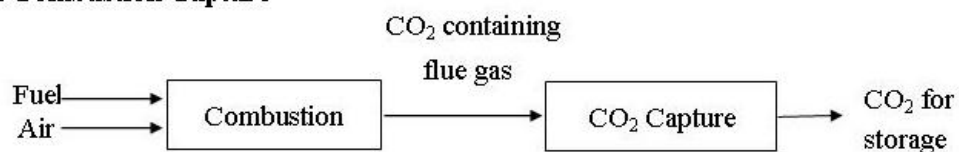
CO<sub>2</sub> capture is applicable to all sources which release large volumes of CO<sub>2</sub>. These include - fossil fuel or biomass energy facilities, major CO<sub>2</sub>-emitting industries, natural gas production, synthetic fuel plants, oil refineries etc. Capturing CO<sub>2</sub> from small sources such as from transportation industries, is difficult and economically not feasible [6]. Therefore, the

development of an efficient and cost-effective CO<sub>2</sub> capture technique is considered to be one of the highest priorities in the field of Carbon capture and sequestration (CCS) [7]. Sequestration strategies adopted so far can be broadly divided into physico-chemical and biological means.

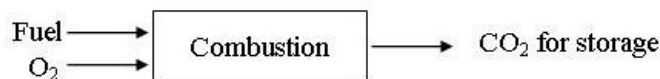
There are 3 basic approaches to CO<sub>2</sub> capture from fossil fuels –

- i) Pre-combustion capture
- ii) Post combustion capture
- iii) Oxy- combustion capture

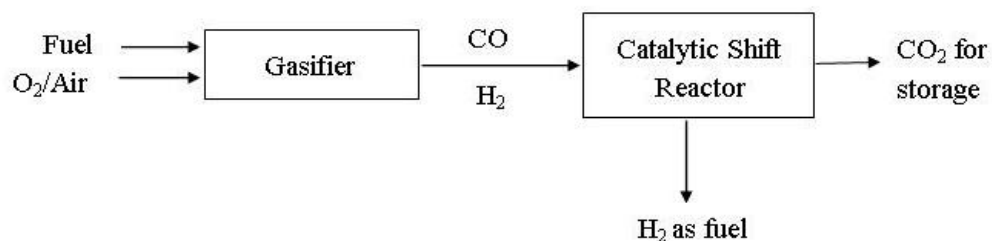
**Post Combustion Capture**



**Oxy Fuel Combustion**



**Pre Combustion Capture**



**Figure 1.2** Different approaches to CO<sub>2</sub> capture – schematic diagram [7]

Post-combustion capture involves removal of CO<sub>2</sub> from flue gas, after combustion by passing it through an equipment which captures CO<sub>2</sub>. Pre-combustion capture CO<sub>2</sub>, as the name suggests is prior to combustion. Oxy-fuel combustion capture involves, burning the fuel in excess of oxygen, instead of air, so that, the flue gas is composed primarily of CO<sub>2</sub> and water vapour, thereby eliminating N<sub>2</sub>, and then removal of CO<sub>2</sub> [8].

Physico-chemical methods for CCS include the following –

#### *Absorption (i.e. solvent scrubbing)*

This is a well-established CO<sub>2</sub> capture system primarily used in the chemical and oil industries. Physical absorption is temperature and pressure dependent with absorption occurring at high pressures and low temperatures. Chemical absorption of CO<sub>2</sub> from gaseous streams such as flue-gases depends on acid–base neutralization reactions using basic solvents. Some of the preferred solvents for CO<sub>2</sub> removal are amines. The released CO<sub>2</sub> is compressed and the regenerated absorbent solution is recycled to the stripper column [7].

#### *Cryogenic distillation*

Cryogenic distillation is an air separation process, where gaseous components of a mixture are separated by condensation. Cryogenic method can capture CO<sub>2</sub> in a liquid form, being easier to transport and storage or send for enhanced oil recovery fields. CO<sub>2</sub> and H<sub>2</sub>O are separated from flue gas on the basis of differences in dew and sublimation points [9].

#### *Membrane Separation*

The operation of membranes is based on the differences in physical or chemical interactions between gases and the membrane material is modified to allow one component to pass through membrane faster than the other. The membrane modules can either be used as conventional membrane separation units or as a gas absorption column. In the former case, CO<sub>2</sub> removal is achieved due to the intrinsic selectivity of the membrane between CO<sub>2</sub> and other gases involved, while in the latter case, CO<sub>2</sub> removal is accomplished by gas absorption where the membranes, usually microporous, hydrophobic and non-selective, are employed as a fixed interface for CO<sub>2</sub> transfer [7].

#### *Adsorption*

Solid adsorbents such as activated carbons, zeolites and mesoporous silicates, alumina, metal oxides have been extensively used for gas separation. The molecular sieving ability of these carbonaceous adsorbents can be controlled during their fabrication process to make them preferentially adsorb a specific gas (e.g. CO<sub>2</sub>) according to the shape and size of the adsorbing molecule [7].

Physical means of CO<sub>2</sub> sequestration has disadvantages, having high costs associated with it thereby need to develop the suitable technologies. Capturing, transporting and storing CO<sub>2</sub>

are also very expensive processes. Biological method of CO<sub>2</sub> sequestration is an alternative to physical methods [10].

Biological CO<sub>2</sub> fixation is currently achieved through the photosynthesis of all terrestrial plants and a tremendous number of photosynthetic microorganisms. Plants are expected to contribute only 3–6% reduction in global CO<sub>2</sub> emissions [11]. However, microalgae and cyanobacteria can grow much faster than terrestrial plants, and their CO<sub>2</sub>-fixation efficiency is about 10–50 times better [12]. The biomass of microalgae and cyanobacteria could also be used as the feed stock for a variety of biofuels, medications, cosmetics and nutritious foods, representing additional benefits from the microalgal CO<sub>2</sub> reduction process. Microalgal- CO<sub>2</sub> fixation is environmentally sustainable when combined with other environmental-protecting processes, such as wastewater treatment or heavy metal removal. A combination of CO<sub>2</sub> fixation, biofuel production, and wastewater treatment may thus provide a very promising alternative to current CO<sub>2</sub> mitigation strategies.

## CHAPTER- 2 LITERATURE REVIEW

### 2.1 Micro-algae for CO<sub>2</sub> sequestration

The use of algae for CO<sub>2</sub> sequestration has several advantages: (i) mitigating CO<sub>2</sub>, the major source of global warming, (ii) producing biofuels and other interesting secondary metabolites, and several others. One kilogram of algal dry cell weight utilizes around 1.83 kg of CO<sub>2</sub>. Annually around 54.9–67.7 tonnes of CO<sub>2</sub> can be sequestered from raceway ponds corresponding to annual dry weight biomass production rate of 30–37 tonnes per hectare [13]. Algal biomass can be used for the production of biofuels (e.g. biodiesel, bioethanol, biohydrogen) and other commercially and scientifically important products like industrial biofilters, food products, and water quality testing [14]. The organic biomass produced through photosynthetic CO<sub>2</sub> fixation could then be used as a renewable biofuel, animal feed or other products [15].

There are several reasons for the greater biomass yields of algae versus land plants. Generally, algae have higher photosynthetic efficiency than land plants because of greater abilities to capture light and convert it to usable chemical energy [16]. Under ideal growth conditions algae direct most of their energy into cell division (6 to 12-hour cycle), allowing for rapid biomass accumulation. Also, unlike plants, unicellular algae do not partition large amounts of biomass into supportive structures such as stems and roots that are energetically expensive to produce and often difficult to harvest and process for biofuel production. In addition, algae have carbon concentrating mechanisms that suppress photorespiration. With algae, all the biomass can be harvested at any time of the year, rather than seasonally. In contrast, only a portion of the total biomass of terrestrial crops (corn cob, soybean seed) is harvested once a year. When algae are grown under stressful conditions (e.g., low nitrogen) or in the presence of supplemental reductants (sugar, glycerol), the metabolism of some species is redirected toward the production and accumulation of energy-dense storage compounds such as lipids [17].

The CO<sub>2</sub> fixation rate is related directly to light utilization efficiency and to cell density of microalgae. Microalgal CO<sub>2</sub> fixation involves photoautotrophic growth in which anthropogenically derived CO<sub>2</sub> may be used as a carbon source. Therefore, biomass measurements or growth rate evaluations are critical in assessing the potential of a microalgal culture system for directly removing CO<sub>2</sub> [18]. Effects of the concentration of CO<sub>2</sub> in airstreams on growth of microalgae in culture have been evaluated in several studies [1, 19]. However, these effects remain to be largely understood.

## 2.2 Algal phylogeny

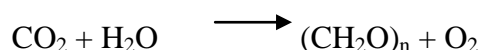
Algae are recognised as one of the oldest life-forms [20]. They are primitive plants (thallophytes), i.e. lacking roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have chlorophyll as their primary photosynthetic pigment. Algal structures are primarily for energy conversion without any development beyond cells, and their simple development allows them to adapt to prevailing environmental conditions and prosper in the long term. The most important classes are: green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta). Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO<sub>2</sub>, salts and a light energy source for growth; while the latter are non-photosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. Some photosynthetic algae are mixotrophic, i.e. they have the ability to both perform photosynthesis and acquire exogenous organic nutrients. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO<sub>2</sub> absorbed by chloroplasts into adenosine triphosphate (ATP) and O<sub>2</sub> the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth [13].

Green algae and cyanobacteria (formally blue-green algae) comprise a vast group of photosynthetic organisms. They are ubiquitously distributed throughout the biosphere and grow under the widest possible variety of conditions from aquatic (freshwater to extreme salinity) to terrestrial places. Its uniqueness that separates them from other microorganisms is due to presence of chlorophyll and having photosynthetic ability in a single algal cell, therefore allowing easy operation for biomass generation and effective genetic and metabolic research in a much shorter time period than conventional plants. Well defined nucleus, a cell wall, chloroplast containing chlorophyll and other pigments, pyrenoid, a dense region containing starch granules on its surface, stigma, and flagella are the major components of green algae [10]. Filamentous colonies of cyanobacteria have ability to differentiate into different cell types like vegetative cells, akinetes, and heterocysts. General function of vegetative cells, akinetes and heterocysts are ability to carry out complete oxygenic photosynthesis, resistance for climate and having a potential to fix nitrogen, respectively. Heterocysts contain the enzyme complex nitrogenase which converts atmospheric nitrogen into ammonium, a unique capacity among photosynthetic oxygenic organisms. These are the

only known prokaryotes having oxygenic photosynthesis for fixation of CO<sub>2</sub> like eukaryotic algae and plants.

### 2.3 CO<sub>2</sub> fixation route

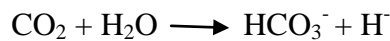
In a multistep process of photosynthesis, plants and algae (green algae and cyanobacteria) fix CO<sub>2</sub> into sugar using light and water as energy and electron source, respectively. The overall reaction for photosynthesis is given by:



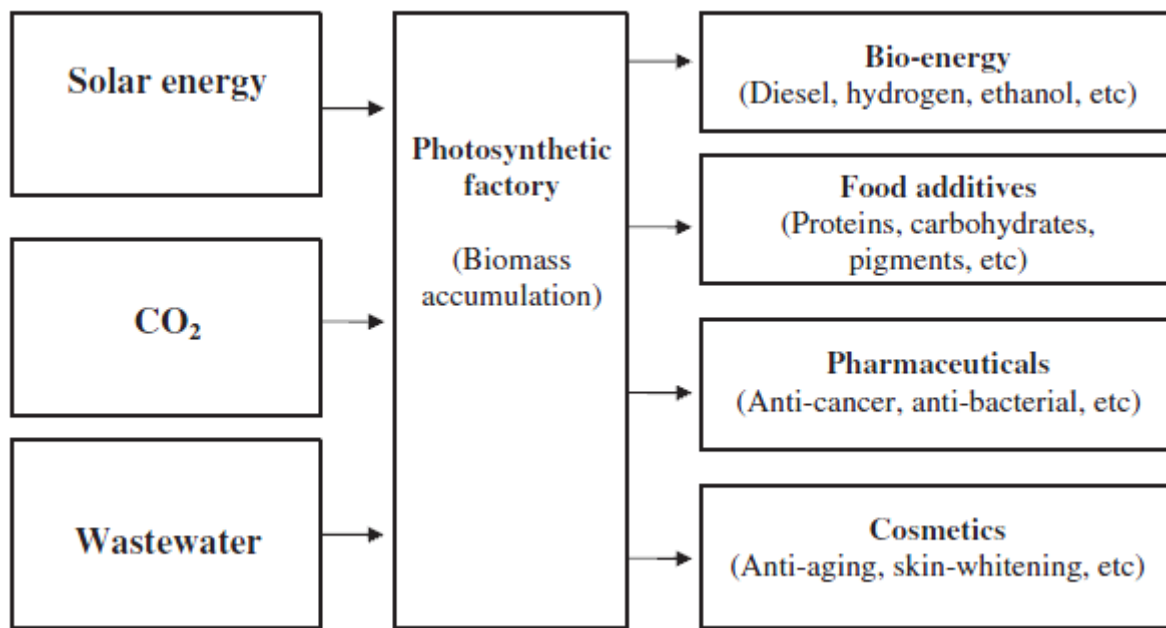
Photosynthesis begins in the photo system II (PS-II) complex, a complicated molecular machine including various cofactors (e.g.  $\beta$ -carotene), and then drives the electron transfer by the excitation of P680 chlorophyll dimer into photo system I (PS-I). Further, PS-I transfers the electrons accepted from PS-II via the P700 dimer of chlorophylls, which are oxidized from light-excited antenna chlorophylls to strongly reducing nicotinamide adenine dinucleotide phosphate (NADPH).

The step of photosynthesis in which CO<sub>2</sub> is converted into sugar with the help of adenosine-50-triphosphate (ATP) by the carboxylase activity of the enzyme ribulose 1,5-bisphosphate carboxylase/ oxygenase (RuBisCO), known as Calvin cycle. Synthesis of one mole CH<sub>2</sub>O, requires a minimum of 8 mol of photons (quanta) each having 218 kJ of energy per mol. Photosynthesis converts approximately 27% of solar energy into chemical energy as it produces 467 kJ of energy per mol of CH<sub>2</sub>O as against 1744 kJ required per mol for its formation [13]. Concentration of CO<sub>2</sub> in water in equilibrium with air is approximately 10  $\mu\text{M}$ . However, since RuBisCO has low affinity for CO<sub>2</sub>, at the normal atmospheric level of CO<sub>2</sub> (390 ppmv) it is only half saturated with the CO<sub>2</sub>. Moreover, it also performs oxygenase activity which produces glycolate 2-phosphate as the end product. It has no use to cell and its synthesis consumes significant amount of cellular energy and also releases previously fixed CO<sub>2</sub> by the carboxylase activity of RuBisCO. The oxygenase activity of RuBisCO inhibits biomass formation of around 50% [21]. To overcome the low affinity of RuBisCO for CO<sub>2</sub>, most algae and cyanobacteria have different CO<sub>2</sub> concentrating mechanisms (CCMs). CCMs activates only at low dissolved carbon concentration. The maximum value of dissolved inorganic carbon till which it is active depends upon strain, pH, light availability, preadaptation of cells etc. For example, in cyanobacteria  $K_m$  (CO<sub>2</sub>) is 200  $\mu\text{M}$  as against approximately 10  $\mu\text{M}$  dissolved CO<sub>2</sub> in water in equilibrium with air. Similarly, in *Chlorella ellipsoidea* at pH 7.5, the minimum equilibrium dissolved inorganic carbon (DIC)

concentration at which high CO<sub>2</sub> characteristics were maintained, i.e. transport was repressed, was 2100 μM, whereas the maximum equilibrium DIC concentration below which DIC transport was fully induced was 500 μM [22]. CCMs acts as an enhancer for higher growth rates in algae and hence can be used for improvement in photobioreactor productivity. The expression of the enzyme carbonic anhydrase (CA) has been associated with induction of the CCMs. CA catalyzes the interconversion of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> and is an important component in the intracellular mobilization of the HCO<sub>3</sub><sup>-</sup> pool, by catalyzing the production of CO<sub>2</sub> for RuBisCO [22].



The overall photosynthetic conversion of CO<sub>2</sub> by microalgae, along with its' possible end products has been summarized in Figure 2.1.



**Figure 2.1** Schematic description of photosynthetic conversion of CO<sub>2</sub>, solar energy, and wastewater into a variety of valuable end products [23]

## 2.4 Growth requirements for micro-algae

Direct utilization of power plant flue gas has been considered for CO<sub>2</sub> sequestration systems. The advantage of utilizing flue gas directly is the reduction of the cost of separating CO<sub>2</sub> gas. Since power plant flue gas contains a higher concentration of CO<sub>2</sub>, identifying high CO<sub>2</sub>

tolerant species is important. Although CO<sub>2</sub> concentrations vary depending on the flue gas source, 15- 20% v/v is typically assumed.

Different parameters need to be considered for selection of micro-algae for sequestration purposes are:

(a) High CO<sub>2</sub> tolerance

Almost all species of green micro-algae, tolerate CO<sub>2</sub> to some extent. Certain strains like *Scenedesmus* sp. has been grown under 80% CO<sub>2</sub> conditions but the maximum cell mass was observed in 10-20% CO<sub>2</sub> concentrations. *Chlorella* sp. can grow under multiple concentrations of CO<sub>2</sub>. High CO<sub>2</sub> tolerance does not necessary translate to better growth or CO<sub>2</sub> uptake. Therefore, other factors need to be taken into account as well before a micro-algae is selected [24-26].

(b) High temperature tolerance

Since the temperature of waste gas from thermal power stations is around 120°C, the use of thermophilic, or high temperature tolerant species are also being considered [27]. Thermophiles can grow in temperature ranging from 42-100°C. An obvious advantage of the use of thermophiles for CO<sub>2</sub> sequestration is reduced cooling costs. In addition, some thermophiles produce unique secondary metabolites, which may reduce overall costs for CO<sub>2</sub> sequestration. Table 2.1 illustrates the temperature and CO<sub>2</sub> tolerance of multiple algal species.

(c) CO<sub>2</sub> assimilation / CO<sub>2</sub> uptake

CO<sub>2</sub> assimilation ability is a pivotal criterion in selecting algae species. Since culture conditions vary from experiment to experiment, comparison is not straightforward.

(d) Light conditions

Light conditions, especially light intensity, are an important factor because the light energy drives photosynthesis. Typical light intensity requirements of microalgae are relatively low in comparison to higher plants. Microalgae often exhibit photoinhibition under excess light conditions. Photoinhibition is often suspected as the major cause of reducing algal productivity. Photoinhibition is a reduction of the photosynthetic activities caused by the exposition to high photon photosynthetic flux density (PPFD). It was well studied and documented for algae as well, when the flux of photons absorbed by chloroplasts is too high,

the concentration of high-energy electrons in the cell is excessive, and they cannot be consumed through the Calvin process. These electrons, on reacting and forming H<sub>2</sub>O<sub>2</sub>, damage the cell structure [28]. Attenuation of light intensity is dependent of its wavelength, cell concentration, penetration distance of light and the geometry of photo-bioreactor. Since the blue and red light are mostly consumed by the microalgae, it penetrates little in microalgae suspension than green light. This effect is more pronounced in the dense culture. In the engineering point of view, geometry of reactor can reduce the attenuation of light in micro algal suspension [29].

Saturation light intensity ( $I_s$ ) is an important parameter which determines the light utilization efficiency and overall photosynthetic efficiency. Pigments present within the photo system are overloaded with the incoming light and interrupt the alteration of light harvesting complex synthesis and degradation. This results in the production of reactive oxygen species causing photo-inhibition and/or photo-oxidative death. Saturation light intensity roughly varies from 30 to 45 W/m<sup>2</sup> with a good estimation [13].

The temperature and CO<sub>2</sub> tolerance of various algal species is given in Table 2.1.

**Table 2.1** Temperature and CO<sub>2</sub> tolerance of various algal species [30]

<b>Algal species</b>	<b>Temperature tolerance (°C)</b>	<b>Maximum CO<sub>2</sub>% (v/v) tolerance</b>
<i>Cyanidium caldarium</i>	60	100
<i>Scenedesmus sp.</i>	30	80
<i>Chlorococcum littorale</i>	–	70
<i>Synechococcus elongates</i>	60	60
<i>Euglena gracilis</i>	–	45
<i>Chlorella sp.</i>	45	40
<i>Chlorella sp. HA-1</i>	–	15
<i>Eudorina sp.</i>	30	20
<i>Dunaliella tertiolecta</i>	–	15
<i>Chlamydomonas sp. MGA 161</i>	35	15
<i>Nannochloris sp.</i>	25	15

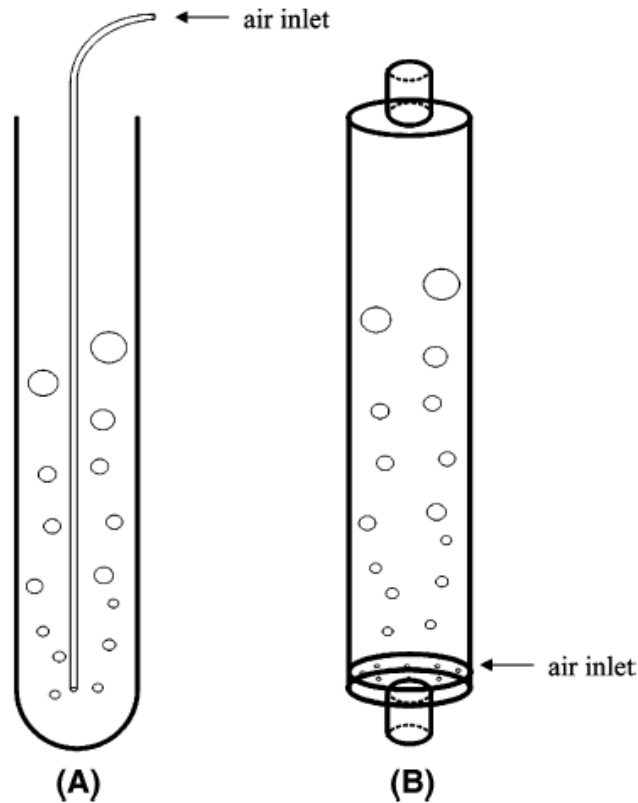
<i>Tetraselmis sp.</i>	-	14
<i>Monoraphidium minutum</i>	25	13.6
<i>Chlorella sp. T-1</i>	35	–

## 2.5 Photo-Bioreactor

Although the term ‘photo-bioreactor’ has been applied to open algal ponds and channels, it is best reserved for devices that allow monoseptic culture which is fully isolated from a potentially contaminating environment. This latter defining convention will be followed here. The available photo-bioreactor configurations are numerous but most may be classified into one of two types: either tubular devices or flat panels. These can be further categorized according to orientation of tubes or panels, the mechanism for circulating the culture, the method used to provide light, the type of gas exchange system, the arrangement of the individual growth units, and the materials of construction employed. Design and scaleup methodologies for photo-bioreactors are poorly developed. Irrespective of the specific reactor configuration employed, several essential issues need addressing (i) effective and efficient provision of light; (ii) supply of carbon dioxide while minimizing losses; (iii) removal of photosynthetically generated oxygen that may inhibit metabolism or otherwise damage the culture if allowed to accumulate (iv) sensible scalability of the photo-bioreactor technology [31].

### 2.5.1 Tubular Reactors

Most configurations of tubular reactors (TR) are one of the following three types: (i) simple airlift and bubble column, which is composed of vertical tubing (in the form of a vertical tubular reactor) that is transparent so as to allow for light penetration and where CO<sub>2</sub> is supplied via bubbling; (ii) horizontal tubular reactor, which is composed of horizontal transparent tubing, usually bearing gas transfer systems attached to the connections; and (iii) helical tubular reactor, which is composed of a flexible plastic tube coiled in a circular framework. Another such reactor that deserves particular attention is the  $\alpha$ -shape tubular reactor, initially conceived by Lee *et al.* [32], because of its unique engineering design that is characterized by a unidirectional, high liquid flow rate, concomitant with a low air flow and an excellent angle relative to sunlight. Air lift and bubble column reactors have been sketched in Figure 2.2.



**Figure 2.2** Schematic representation of airlift (A) and bubble column (B) reactors. (Vertical Tubular Reactors) [33]

### 2.5.1.1 Vertical tubular reactors

The airlift and bubble column reactors are examples of vertical tubular reactors (VTR), regularly composed of polyethylene or glass tubes, which are sufficiently transparent to allow good light penetration but are manufactured with sufficiently common materials so as to be non-expensive. Air is bubbled at the bottom - a strategy that provides good overall mixing, sufficient supply of  $\text{CO}_2$ , and efficient removal of  $\text{O}_2$ . Polyethylene bags have frequently been used, with advantage taken from their particularly low cost, high transparency and good sterility at startup-due to the high temperatures used during film extrusion. More recently, Chae *et al.* [1] reported a pilot-scale photo-bioreactor that uses sunlight and flue gas, and consists of a vertical tubular part (kept in the dark) and a horizontal tubular part (subject to sunlight). Although cultivation of microalgae in the above systems is simple and hence widely employed (including in hatcheries), the corresponding technology is somehow primitive, with obvious constraints derived from the high fragility and the low versatility of the material in stake. Furthermore, scale-up of these systems was initially thought to be easy, but accumulated experience has indicated that increases in culture volume decrease bag

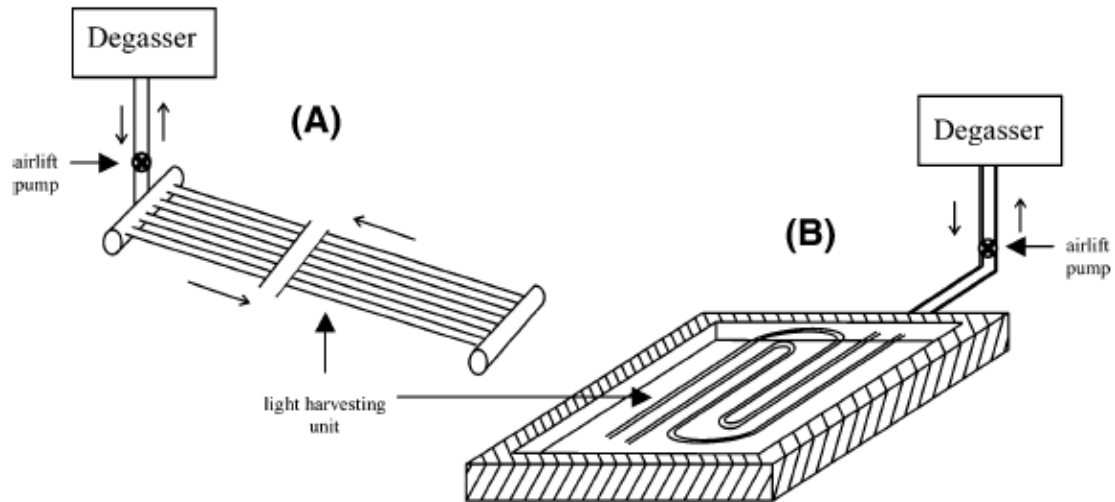
productivity. Rigid VTRs have also been frequently used. A 33.7 cm X 250 cm (ca. 40 L) polyethylene reactor was discussed by Laing and Jones [34], in which temperature was controlled by a refrigeration fluid flown through its double wall and in which artificial light was provided from the inside. Scale-up is not straightforward; furthermore, in order to provide enough culture volume, as well as efficient gas transfer rate, the reactor diameter should be relatively high when compared to flat plate or tubular loop reactors, a requirement that in turn decreases the area-to-volume ratio and consequently constrains photosynthetic efficiency. Another major drawback is the large angle relative to the direction of sunlight, which causes a high fraction of incident.

#### **2.5.1.2 Horizontal tubular reactors**

Horizontal tubular reactors (HTR) have been on the rise; gas transfer takes place in the tube connection or via a dedicated gas-exchange unit, and the angle toward sunlight is particularly adequate for efficient light harvesting. Such systems can handle large working volumes, because they are not susceptible to contamination. On the other hand, they may generate considerable amounts of heat, which may attain temperature amplitudes of 20 °C within a single day if (costly) temperature control systems are not provided; thus, it will likely pose a problem for regular operation [33].

Another advance in microalgal cultivation systems is the near horizontal tubular reactor (NHTR) designed by Tredici and Zetelli, [35] which consists of sets of parallel tubes made of flexible plastic (typically 6.4 m in length, 43 mm diameter and 0.15 mm thick), connected by PVC (polyvinyl chloride) manifolds. The upper manifolds are used as degassers, and a perforated pipe inserted in the lower manifolds is used to inject air in each individual tube. The tubes are placed at an angle of 5-7° relative to the horizontal plan. Temperature is controlled by activating water spraying onto the reactor when the culture temperature exceeds a preset value. The maximum volume tested, 4000 L, obtained with a set of 8 parallel tubes 44 m in length, was associated with a mean productivity of 0.7 g L<sup>-1</sup> day<sup>-1</sup> in the case of *Nannochloropsis* sp. The main advantage of this system is the high area-to-volume ratio and its easy scale-up, coupled with the possibility for a higher degree of control. Surprisingly, the temperature control was not efficient, and a day-night temperature gap of ca. 14 °C during summer and of ca. 22 °C during spring was actually recorded. Such a poor control led to different biochemical compositions of the microalga throughout the year. A basic outline of horizontal tubular reactors is given in Figure 2.3. However, the major drawback of this

system was probably its low gas transfer rate, arising from a large length and a small diameter.

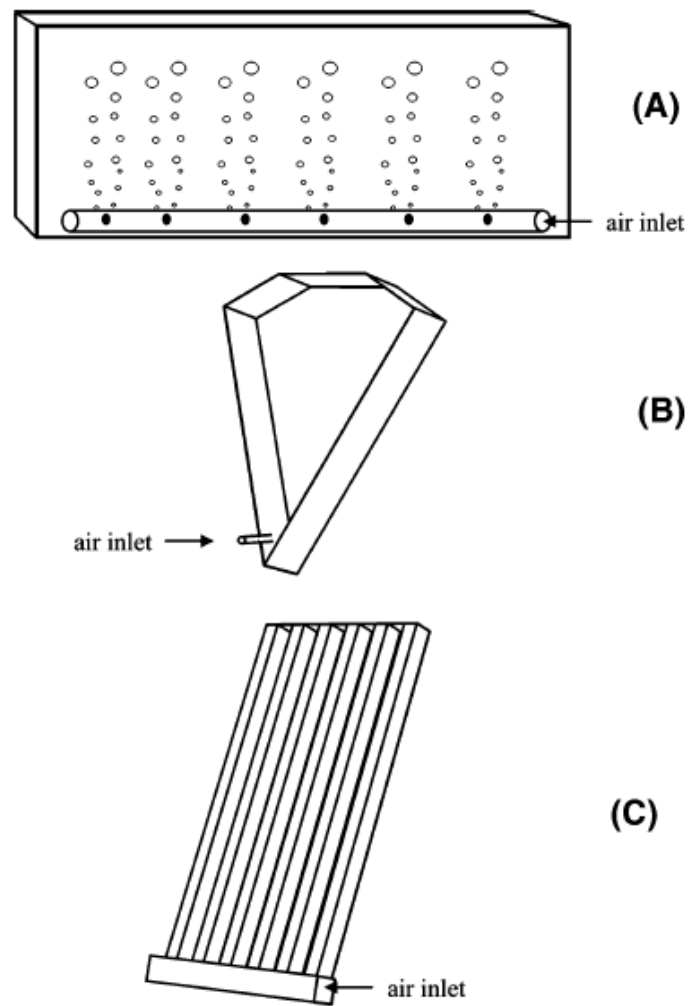


**Figure 2.3** Schematic representation of horizontal tubular reactor with a degassing unit and a light harvesting unit, composed of parallel sets of tubes (A) or a loop tube (B) [33]

### 2.5.2 Flat plate reactors (FPR)

Flat plate reactors (FPR) are conceptually designed to make efficient use of sunlight; hence, narrow panels are usually built so as to attain high area-to volume ratios. In the early 1980s, FPR were considered expensive and were even claimed to exhibit deficiencies in culture flow control. A 500-L FPR was developed by Pulz [36], in which the culture was circulated from an open gas exchange unit through several parallel panels placed horizontally. The culture flew at a high linear speed (viz.,  $1.2 \text{ m s}^{-1}$ ), but hydrodynamic parameters usually lay in a safe operating range for the sake of cell integrity. Introduction of alveolar panels, made of PVC, polycarbonate or polymethyl methacrylate, for microalga cultivation has meanwhile emerged as a successful concept, because of their high versatility and commercial availability. The greatest advantage of this system is its provision of an open gas transfer unit, which has proven efficient in overcoming the problem of oxygen buildup; however, such an open zone restricts effectiveness of contamination control, as compared with completely closed reactors. A schematic representation of FPRs is given in Figure 2.4. In general, the main advantages of FPR are their high productivity and uniform distribution of light and, in the specific case of bubbled column FPR, the absence of a driving pump. Furthermore, these reactors can be

oriented toward the sun, hence permitting a better efficiency in terms of energy absorbed from incident sunlight. The main disadvantage of alveolar panels is oxygen buildup, which arises from the high photosynthetic activity reached, coupled with the small diameter of the reactors used.



**Figure 2.4** Schematic representation of flat panel reactors: flat panel bubbled in the bottom (A), V-shaped panel (B) and alveolar panel (C) [33]

### 2.5.3 Fermenter-Type Reactors

The least expanded systems for microalga cultivation are conventional fermenter-type reactors (FTR). These systems present indeed an intrinsic disadvantage: the area-to-volume ratio is quite low, so sunlight harvesting efficiency is poor. To overcome this nuclear drawback, sophisticated systems of internal illumination were developed, which are able to provide a more homogeneous distribution of light. When possible, microalgae may be heterotrophically cultivated in FTR, using appropriate organic carbon sources. Several laboratory-scale FTR have been developed, but there is scarce information available on their large-scale counterparts. The main advantage of these systems is of course the accurate control of processing parameters, including light, coupled with the vast experience accumulated in food and pharmaceutical industries in terms of the scale-up thereof. Therefore, should productivity be enhanced, FTR would certainly become a competitive alternative for industrial manufacture of biochemical products brought about by microalgae. [33]. The advantages and disadvantages of different closed photo-bioreactor systems are illustrated in table 2.2.

**Table 2.2** Advantages and disadvantages of different closed photobioreactor systems for algal cultivation

<b>Photobioreactor system</b>	<b>Advantages</b>	<b>Disadvantages</b>
Tubular PBRs	<ul style="list-style-type: none"> <li>• Large illumination surface area</li> <li>• Fairly high biomass productivity</li> <li>• Relatively cheap</li> <li>• Suitable for outdoors</li> </ul>	<ul style="list-style-type: none"> <li>• Fouling</li> <li>• Large area of land needed</li> <li>• Poor mass transfer</li> <li>• High O<sub>2</sub> accumulation</li> <li>• Photo-inhibition risk</li> <li>• Hard to control temperature</li> </ul>
Flat-plate PBRs	<ul style="list-style-type: none"> <li>• Huge illumination surface area</li> <li>• Good light path</li> <li>• High biomass productivity</li> <li>• Easy to scale up</li> <li>• Suitable for outdoors</li> <li>• Relatively low O<sub>2</sub> accumulation</li> <li>• High photosynthetic efficiency</li> </ul>	<ul style="list-style-type: none"> <li>• High hydrodynamic stress</li> <li>• Hard to control temperature</li> </ul>
Column PBRs	<ul style="list-style-type: none"> <li>• Low shear stress</li> <li>• Low energy consumption</li> <li>• Cheap and easy operation</li> </ul>	<ul style="list-style-type: none"> <li>• Small illumination surface area</li> </ul>

## 2.6 CO<sub>2</sub> capture studies using *Chlorella* species

*Chlorella* species have been long reported to have successfully fixed high CO<sub>2</sub> levels as well as being tolerant to high levels of CO<sub>2</sub> [10]. Moreover, since *Chlorella* strains tend to have high lipid content, they are potential biofuel sources as well.

A study was conducted in the Lafarge Perlmooser cement plant in Retznei, Austria, using *Chlorella emersonii*. During 30 days of cultivation, flue gas had no visible adverse effects compared to the controls grown with pure CO<sub>2</sub>. The semi-continuous cultivation with media recycling was performed in 5.5-L pH-stat photo-bioreactors. The assay using CO<sub>2</sub> from flue gas yielded a total of 2.00 g L<sup>-1</sup> microalgal dry mass and a CO<sub>2</sub> fixation of 3.25 g L<sup>-1</sup>. In the control, a total of 2.06 g L<sup>-1</sup> dry mass was produced and 3.38 g L<sup>-1</sup> CO<sub>2</sub> was fixed. No accumulation of flue gas residues was detected in the culture medium[37].

In another study carried out at National Chiao Tung University, Taiwan the marine microalga *Chlorella* sp. was cultured in a photo-bioreactor to assess biomass, lipid productivity and CO<sub>2</sub> reduction. They also determined the effects of cell density and CO<sub>2</sub> concentration on the growth of *Chlorella* sp. During an 8-day interval cultures in the semi-continuous cultivation, the specific growth rate and biomass of *Chlorella* sp. cultures in the conditions aerated 2–15% CO<sub>2</sub> were 0.58–0.66 day<sup>-1</sup> and 0.76–0.87 g L<sup>-1</sup>, respectively. At CO<sub>2</sub> concentrations of 2%, 5%, 10% and 15%, the rate of CO<sub>2</sub> reduction was 0.261, 0.316, 0.466 and 0.573 g hour<sup>-1</sup>, and efficiency of CO<sub>2</sub> removal was 58%, 27%, 20% and 16%, respectively. The efficiency of CO<sub>2</sub> removal was similar in the single photo-bioreactor and in the six-parallel photo-bioreactor. However, CO<sub>2</sub> reduction, production of biomass, and production of lipid were six times greater in the six-parallel photo-bioreactor than those in the single photo-bioreactor. Thus, inhibition of microalgal growth cultured in the system with high CO<sub>2</sub> (10–15%) aeration could be overcome via a high-density culture of microalgal inoculum that was adapted to 2% CO<sub>2</sub> [18].

A study at Indian Institute of Technology, Kharagpur investigated the feasibility of bioCO<sub>2</sub> sequestration using *Chlorella sorokiniana*. It was found that 5% CO<sub>2</sub> (v/v) in air was the most suitable concentration for the growth of this organism. At this concentration, the maximum rate of CO<sub>2</sub> sequestered and the biomass obtained were found to be 1.21 gL<sup>-1</sup> day<sup>-1</sup> and 4.4 gL<sup>-1</sup> respectively. Additionally, the study evaluated the performance of two reactors namely: bubble column and airlift reactor based on their growth profile and transport properties like

$k_{La}$  and mixing time. The growth profile was better in airlift reactor and it provides cyclic axial mixing of media [38].

In other studies, different concentrations of  $CO_2$  were added to cultures *Chlorella kessleri*, *C. vulgaris* and *Scenedesmus obliquus*, and *Spirulina* sp., growing in flasks and in a photo-bioreactor. In each case, the best kinetics and carbon fixation rate were with a vertical tubular photo-bioreactor. *C. Vulgaris* was seen to tolerate up to 18%  $CO_2$  [39].

The effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris* was observed at Durban University of Technology, South Africa. The optimized conditions for higher biomass yield of the selected strain were at 4%  $CO_2$ ,  $0.5\text{ g l}^{-1} NO_3^-$  and  $0.04\text{ g l}^{-1} PO_4^{3-}$ , respectively. The pulse amplitude modulation results indicated that *C. vulgaris* could withstand a light intensity ranging from 150 to 350  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . Further increase in light intensity resulted in a decline of the electron transport rate. Carbon fixation rate, lipid content and calorific value of *C. vulgaris* was  $6.17\text{ mg l}^{-1}\text{ hour}^{-1}$ , 21% and  $17.44\text{ kJ g}^{-1}$ , respectively. The pyrolytic studies under inert atmosphere at different heating rates of 15, 30, 40 and  $50^\circ\text{C min}^{-1}$  from ambient temperature to  $800^\circ\text{C}$  showed that the overall final weight loss recorded for the four different heating rates was in the range of 78.9-81%. These studies could be useful to appraise the biofuel potential of the isolated *C. vulgaris* strain, which can later be taken for pilot scale production [40].

## CHAPTER-3 MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 *Microorganisms and culture medium*

The phototroph used was the microalga *Chlorella vulgaris*. The strain was obtained from Science Technology and Entrepreneur's Park, Thapar University Patiala. The medium used for maintenance of the inoculum as well as for cultivation for the purpose of CO<sub>2</sub> capture studies, was Fogg's Medium [41]. Composition of Fogg's medium is given in Table 3.1.

**Table 3.1** Fogg's medium composition

Ingredients	Concentration
Potassium nitrate	0.5 g/L
Di-potassium hydrogen phosphate	0.2 g/L
Magnesium sulphate	0.2 g/L
Calcium chloride	0.1 g/L
A5 micronutrient solution	1mL/L
Fe-EDTA stock solution	1 mL/L

The A5 micronutrient solution as the name suggests contains traces of metallic ions in the form of salts and other minerals. Certain ions and salts are needed in minute amounts. A5 micronutrient solution is added to meet this requirement. Composition of this solution is given in Table 3.2.

**Table 3.2** A5 micronutrient composition

Ingredients	Concentration
Boric acid	2.86 g/L
Manganese chloride	1.81 g/L
Zinc sulphate	0.22 g/L
Sodium molybdate	0.018 g/L
Copper sulphate	0.08 g/L

All chemicals were of AR grade and obtained from Himedia, Loba Chemie and SD Fine Chemicals.

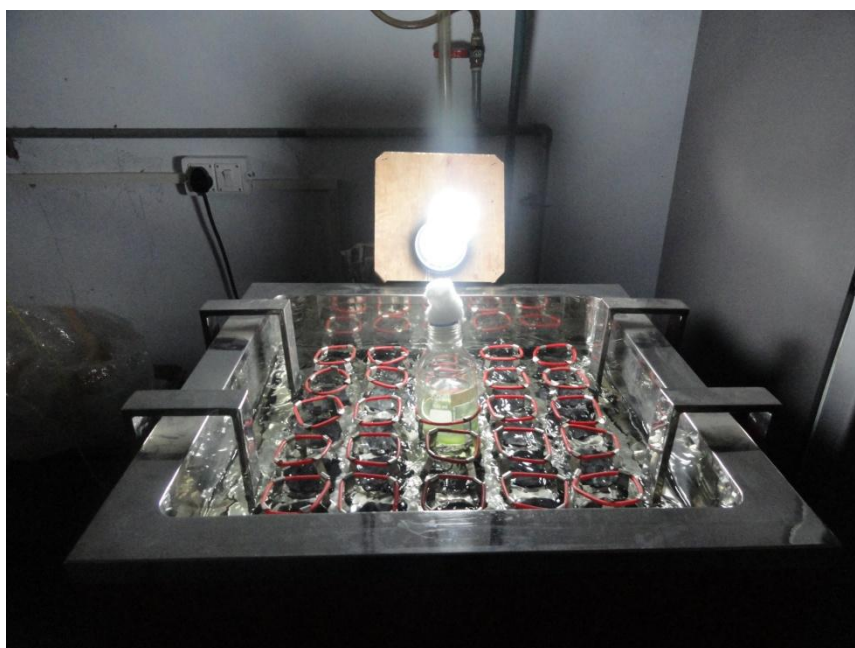
#### 3.1.2 *Light source*

Lighting was provided with the help of 85 Watts cool white fluorescent light of CGL make.

## 3.2 Instruments/ Equipments

### 3.2.1 Set-up for sub-culturing

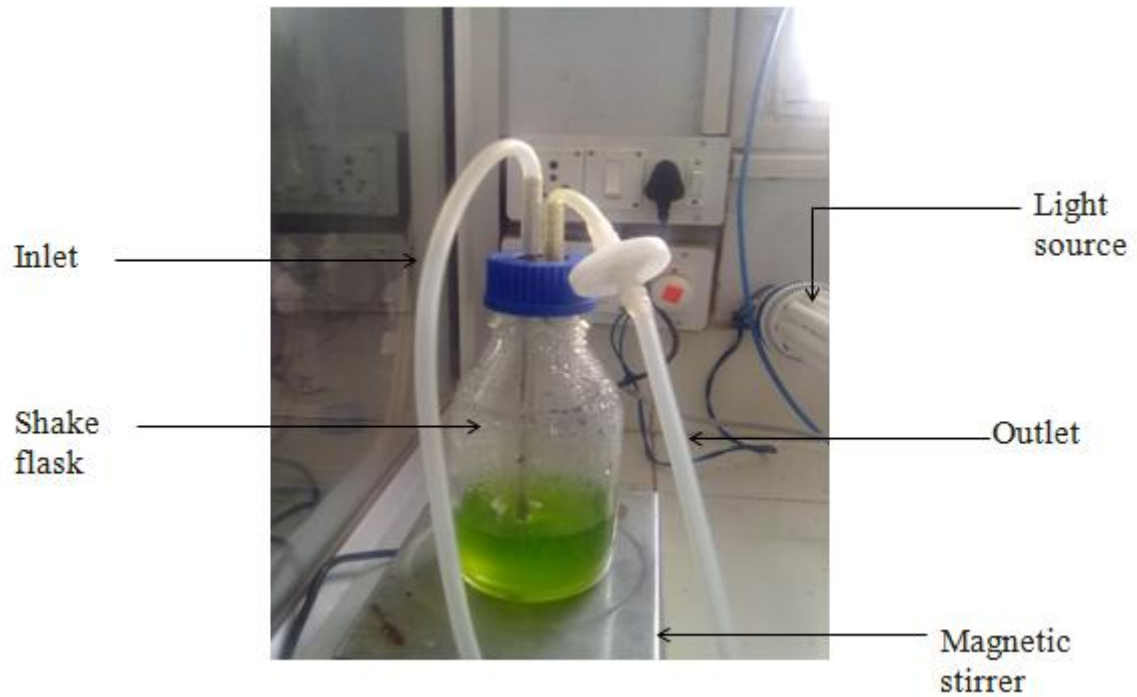
The set-up for sub-culturing included a light source, and a temperature controlled orbital shaker. Flasks containing the media as well as the inocula were placed in the orbital shaker. A diagram of the setup is provided in Figure 3.1.



**Figure 3.1** Set up for sub-culturing

### 3.2.2 Set up for CO<sub>2</sub> fixation

Set up for the CO<sub>2</sub> fixation experiments consisted of a single light source, a magnetic stirrer, a shake flask containing inoculum and media and a CO<sub>2</sub>/air gas supply. CO<sub>2</sub> was provided by a CO<sub>2</sub> gas cylinder of 20 L capacity obtained from Lalit Gases, Patiala. Air was supplied through an oil-free air compressor of Apcon, India make. Figure of the experimental setup is given in Figure 3.2. The gas flow was controlled with the help of a needle valve and a soap-bubble flow meter.



**Figure 3.2** Experimental set up for CO<sub>2</sub> fixation experiments

### 3.2.3 UV-Vis spectrophotometer

Absorbance of algal cell suspension was taken at regular intervals, at 680 nm by a Lambda 35 UV/Visible Spectrophotometer (Perkin Elmer, USA) (Figure 3.3).



**Figure 3.3** UV-Visible spectrophotometer

### 3.2.4 Gas chromatograph

Inlet and outlet gas concentrations were measured with the help of Gas Chromatograph, of model 7890 A series purchased from Agilent Technologies, India. Operating conditions were:

- Inlet temperature 180 °C
- Front detector temperature 200 °C
- Oven/column temperature 60 °C
- Column flow rate 3 mL min<sup>-1</sup>



**Figure 3.4** Gas chromatograph

### 3.2.5 TOC analyser

Total organic carbon (TOC) was measured with the help of TOC-V<sub>CPH</sub> Total organic carbon analyzer solid sampling module (Shimadzu, Japan) as shown in Figure 3.5.



**Figure 3.5** TOC analyser

### **3.2.6** *Particle Size Analyser*

Size distribution of the algal cells was obtained by photon correlation spectroscopy. The measurements were carried out on Brookhaven 90 plus Particle Size Analyzer at 25°C.

### **3.2.7** *Microscope*

Images of live algal cells, were captured using a Nikon eclipse 50 i microscope at 40X and 100X magnifications.

### **3.2.8** *Centrifuge*

Remi, R- 8C, BL centrifuge was used for phase separation of micro-algal cells and growth media.

### **3.2.9** *Autoclave*

Autoclave obtained from Equitron, India was used for sterilization of media (Figure 3.6).



**Figure 3.6** Autoclave

### **3.2.10** *pH meter*

pH meter was obtained from Thermo Scientific, Orion 5-Star model. This was used to check the pH of the media and to make adjustments in the pH, when necessary, with the help of 0.1 N HCl and 0.1N NaOH.

### **3.2.11** *Lux meter*

Lux meter was obtained from Mastech, India, model MS 6610 to monitor the light intensity provided to the algal culture.

### **3.2.12** *Laminar air Flow Chamber*

Laminar Air Flow chamber from Thermodyne Pvt. Ltd., Faridabad (Figure 3.7) was used for transferring the culture in sterile conditions.



**Figure 3.7** Laminar Air Flow Chamber

### **3.3 Experimental Procedure**

#### **3.3.1 *Sub-culturing***

Sub-culturing was carried out for 5 days with the inocula, in shake flasks containing appropriate media. The flasks were plugged and not aerated. Light intensity in the range of 1950-2750 lux was provided with the help of 85 Watt CFL. Initial pH of the media was set to 7.

#### **3.3.2 *CO<sub>2</sub> fixation experiments***

Micro-algal cells were cultured in shake flasks with a working volume of 200 mL. A surface light intensity in the range of 1975 – 2900 lux was provided by 85 W continuous, cool-white, fluorescent bulbs. Gas supply at 0.5 v/v/m was supplied through oil free air compressor and CO<sub>2</sub> cylinder. CO<sub>2</sub> volumetric fraction was fixed at 10%. The flow rates of CO<sub>2</sub> and air was controlled and monitored with the help of needle valve and soap bubble meter.

### 3.3.3 Measurement of microalgal cells growth rate

The biomass concentration ( $\text{g L}^{-1}$ ) was determined according to the method previously reported [42]. Regression equations of the relationship between optical density and cell dry weight were established. Each sample was diluted to give an absorbance in the range of 0.1–1.0 if optical density was greater than 1.0. The optical density was used to evaluate the biomass concentration of *Chlorella vulgaris* in each experiment. In the present study, we used biomass concentration for the quantification of *Chlorella vulgaris* in the culture. To obtain the dry cell weight, microalgal cells were collected, and centrifuged. The washed microalgal pellets were dried at 75 °C for 24 h; afterward, in a hot-air oven and the dried cells were for dry weight measurement. The growth rate was measured according to the equation showed as follows:

$$\text{Growth rate} = \frac{W_f - W_i}{D_t}$$

where  $W_f$  and  $W_i$  is the final and initial biomass concentration, respectively.  $D_t$  is the cultivation time in days. A line diagram illustrating all the steps involved in the CO<sub>2</sub> fixation experiments is given in Figure 3.8.

### 3.3.4 Chemical analyses

The outlet load of gas streams were monitored with the help of a gas chromatograph. The concentration of CO<sub>2</sub> in the inlet and outlet gas streams were measured using 7890 A Series, Gas chromatograph (Agilent Technologies, US). Samples were collected in glass balloon and immediately injected via gas tight syringe into the gas chromatograph.

CO<sub>2</sub> bio-fixation ratio was calculated using the following equation.

$$\eta = \frac{y_{inlet} - y_{outlet}}{y_{inlet}}$$

$y_{inlet}$  and  $y_{outlet}$  are concentrations in the inlet and outlet gas streams and  $\eta$  is CO<sub>2</sub> biofixation ratio.

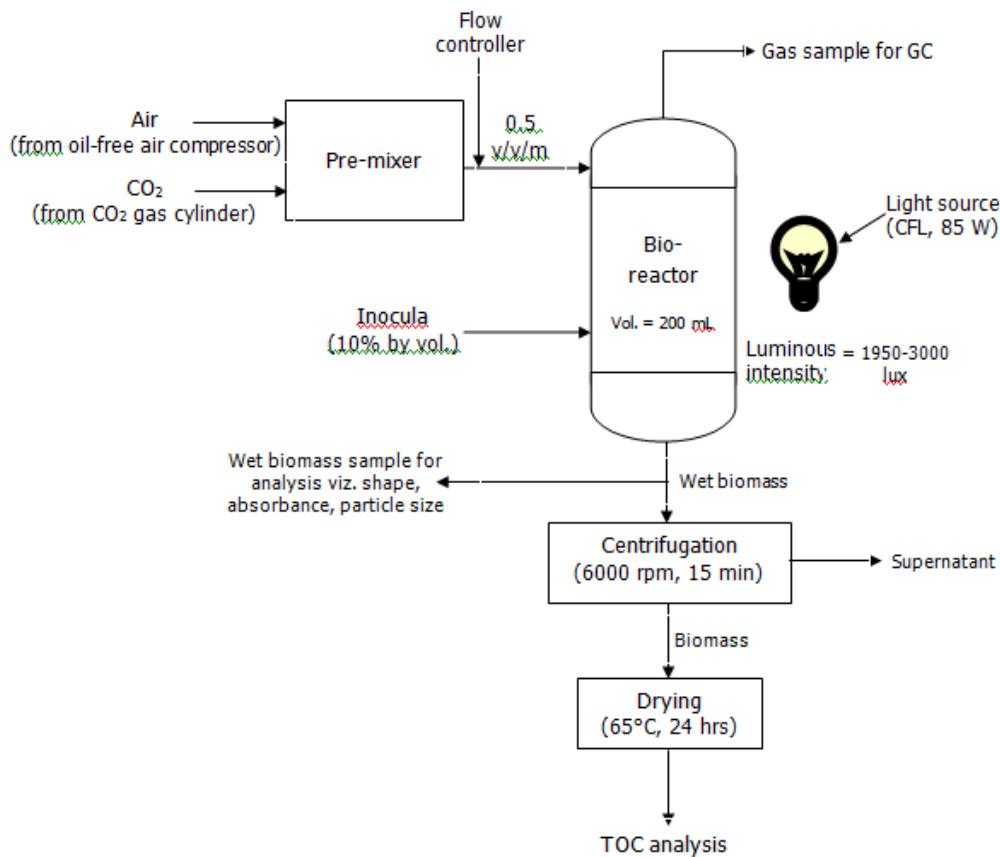
Total organic carbon (TOC) was measured with the help of TOC-V<sub>CPH</sub> Total organic carbon analyzer solid sampling module (Shimadzu, Japan). TOC was then co-related to CO<sub>2</sub> uptake using the following equation:

$$\text{CO}_2 \text{ uptake} = X \times \frac{M_{\text{CO}_2}}{M_C}$$

Where X is the biomass concentration (g L<sup>-1</sup>), M<sub>CO<sub>2</sub></sub> is molecular weight of CO<sub>2</sub> and M<sub>C</sub> is molecular weight of carbon. CO<sub>2</sub> uptake rates can be calculated by –

$$\text{CO}_2 \text{ uptake rate} = \frac{\text{CO}_2 \text{ uptake}}{\text{time}}$$

CO<sub>2</sub> uptake rate and CO<sub>2</sub> capture efficiency are then related to growth rates and studied further.

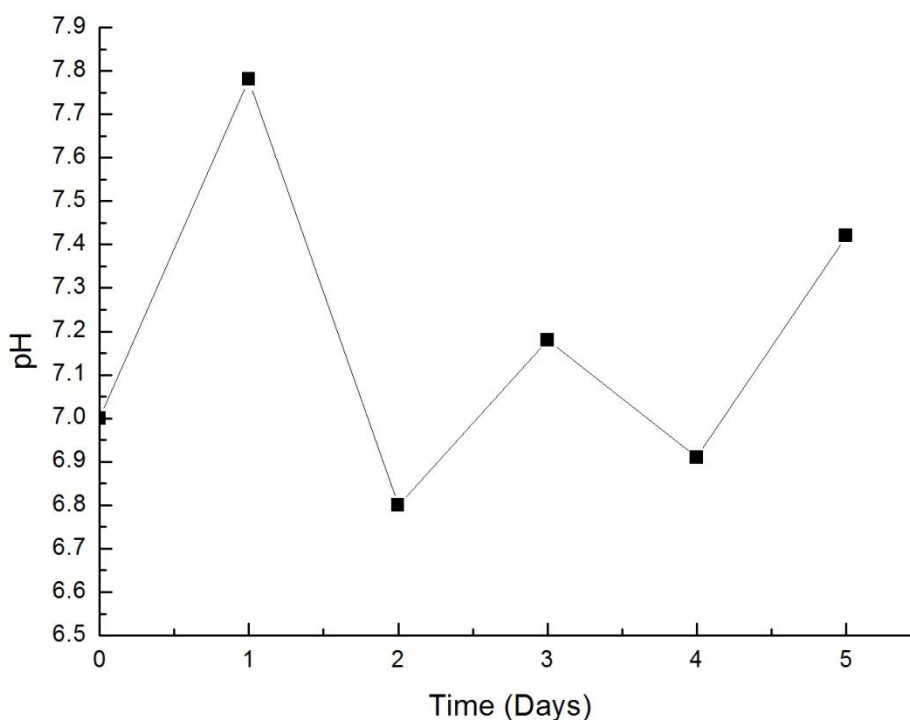


**Figure 3.8** Line diagram for steps incurred in CO<sub>2</sub> fixation

## CHAPTER -4 RESULTS AND DISCUSSION

### 4.1 Micro-algal growth conditions

Micro-algal growth as mentioned earlier, is dependent on a lot of factors, primarily temperature, pH, light intensity, and CO<sub>2</sub> percentage. Optimum light intensity, obtained from literature for growth of *Chlorella vulgaris*, was 2000 – 4000 lux and this was maintained. Temperature was not controlled, but was monitored thrice a day. The approximate temperature variation was 28 to 35 degrees Celsius, over the course of our experiments. This is slightly, higher than the optimum temperature prescribed for *Chlorella* growth. As our CO<sub>2</sub> experiments were started on the onset of summer, the ambient temperature rose every day. However, this did not affect the growth rate of *Chlorella* appreciably. Flue gases contain low amounts of CO<sub>2</sub> (8-20%). A volumetric fraction of 10% was kept keeping this in mind. Initial pH was kept at 7 and the values fluctuated over time, but not much. pH variations over the course of 5 days, is shown in Figure 4.1.



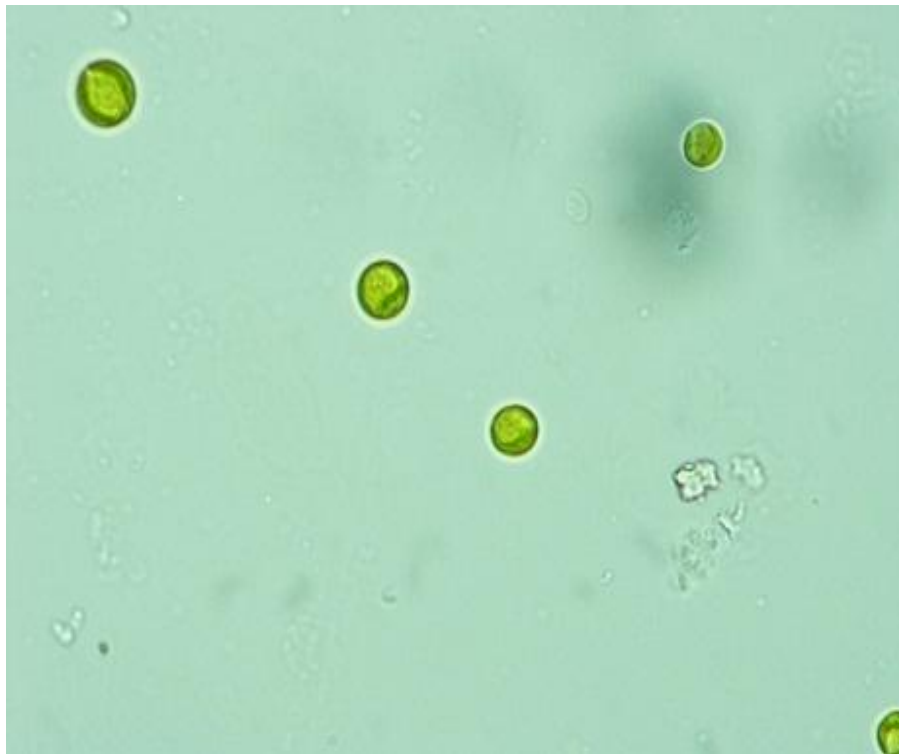
**Figure 4.1** Variation of pH over the cultivation period

Most *Chlorella* sp. are known to have retarded or no growth between acidic (3.0-6.2) and alkaline values (8.3-9.0). pH values were seen to fluctuate between 7.8 and 6.9. Unutilised CO<sub>2</sub> at elevated concentration will be converted to H<sub>2</sub>CO<sub>3</sub> which leads to a fall in pH. Often,

if CO<sub>2</sub> concentration in gaseous form is lower than what is required, algae begin to use CO<sub>2</sub> in aqueous form as compensation. This corresponds to an increase in pH.

#### 4.2 Physical and morphological characteristics of *Chlorella vulgaris*

Slides containing the algal strain were viewed at 50X and 100 X resolution under a microscope. The pictures obtained at 100X are given in Figure 4.2.



**Figure 4.2** Algal cells at 100 X magnification

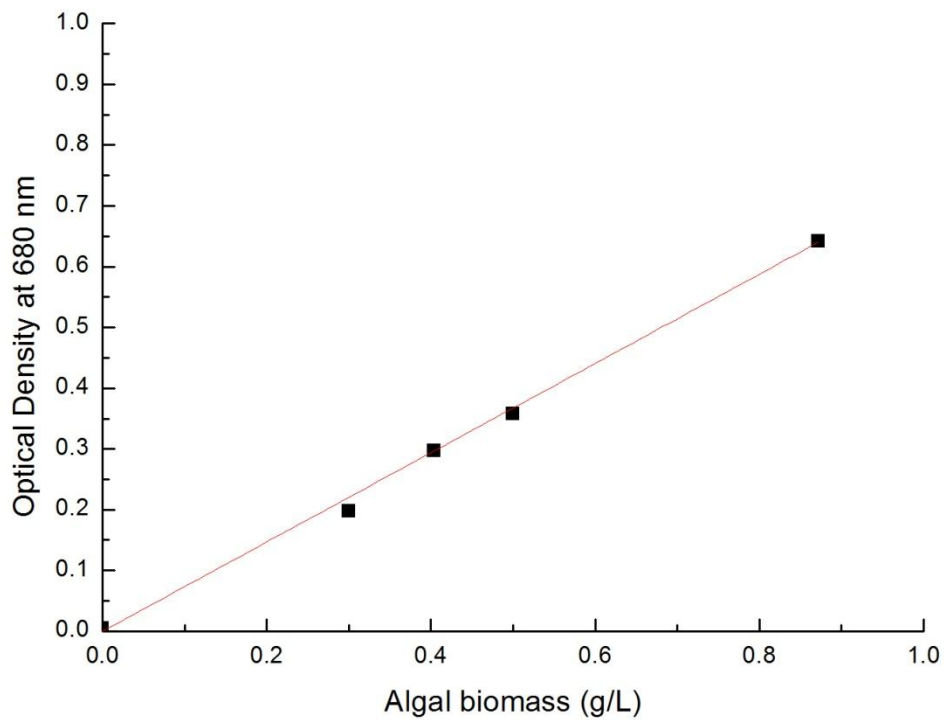
The image depicts a spherical shape of the cells. *Chlorella vulgaris* is seen to be uni-cellular, green with minimal cluster formation. Average hydrodynamic size of the cells was determined by photon correlation spectroscopy and the mean hydrodynamic size and polydispersity indices were determined. Effective hydrodynamic diameter was obtained to be 2.37  $\mu\text{m}$  with very low polydispersity. Low polydispersity, indicates formation of single domains, or in other words, no cluster formation.

### 4.3 *Chlorella vulgaris* dry weight calibration curve

Regression equations of the relationship between optical density and cell dry weight were established and shown as follows:

$$y=0.73471x; R^2 = 0.99848$$

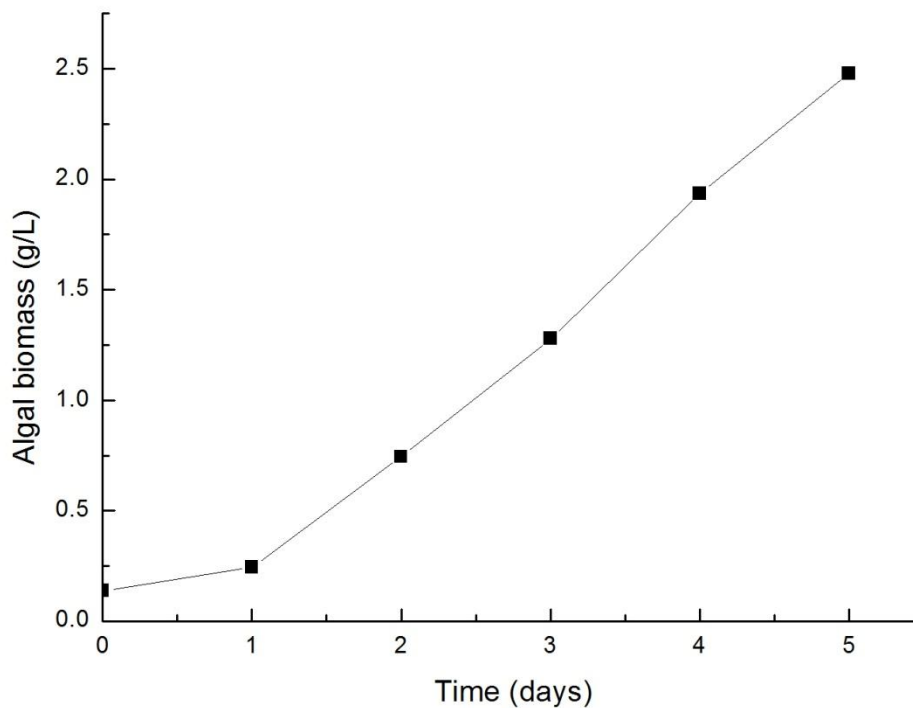
The value x is biomass concentration ( $\text{g L}^{-1}$ ). The value y is optical density of biomass measured by UV-visible spectrophotometer at 680 nm. Graphical representation of the calibration curve is given in Figure 4.3.



**Figure 4.3** Algal biomass calibration curve

#### 4.4 Growth kinetics for *Chlorella vulgaris*

*Chlorella vulgaris* is a robust strain. It has been reported to grow under very adverse conditions. The growth pattern of *Chlorella vulgaris* during our experiments is depicted in Figure 4.4.

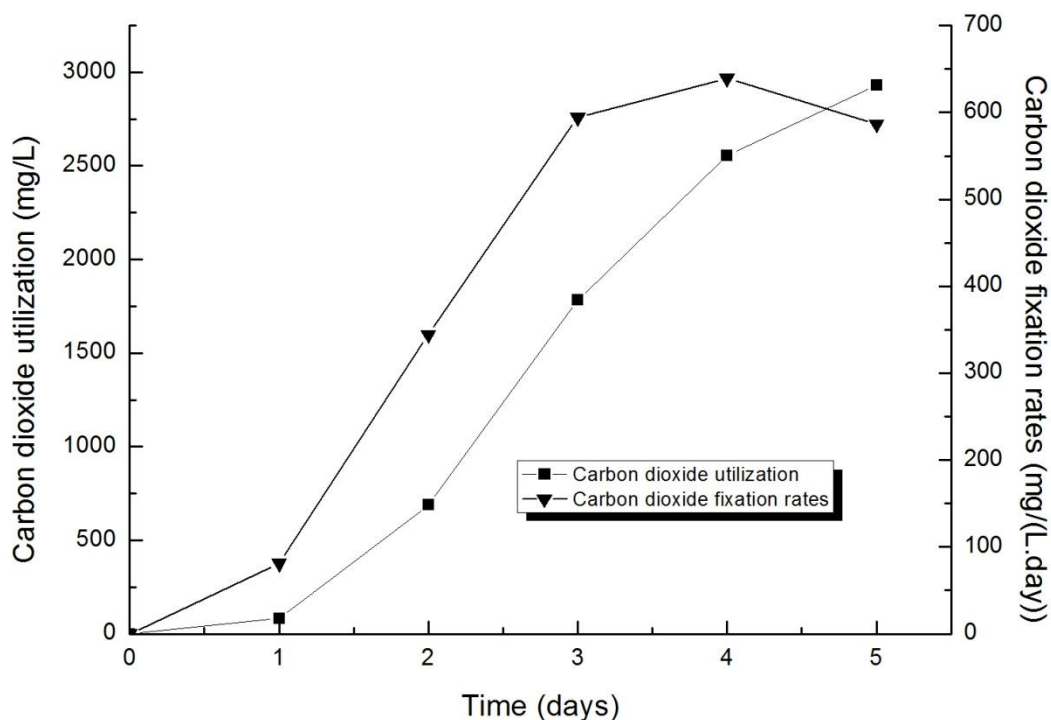


**Figure 4.4** Growth kinetics of *Chlorella vulgaris* with 10% CO<sub>2</sub>

A small lag phase is seen up to day 1. This can be attributed to the fact that the culture was acclimatising to the CO<sub>2</sub> rich conditions. Air contains 0.00397%, volumetric fraction of CO<sub>2</sub> while flue gases contain anywhere between 10-20%. During sub-culturing, no CO<sub>2</sub> was provided separately, hence the inocula used for the CO<sub>2</sub> fixing experiments required an adjustment period before it began to use CO<sub>2</sub> efficiently. After the initial lag phase, the microalgal strain exhibited an almost linear growth pattern over a period of 5 days. The growth rate observed for *Chlorella vulgaris* was 0.596155 mg L<sup>-1</sup> day<sup>-1</sup>. It can be inferred from the curve that the growth is still in logarithmic phase after 5 days, i.e. culture is healthy and is still growing. This is slightly higher than reported values [40].

#### 4.5 CO<sub>2</sub> fixation and fixation rates

The CO<sub>2</sub> fixation patterns depict a trend almost similar to what is seen, in terms of growth activity of the micro-algal strain. Figure 4.5 depicts the carbon fixation as well as its' rates with respect to cultivation time.



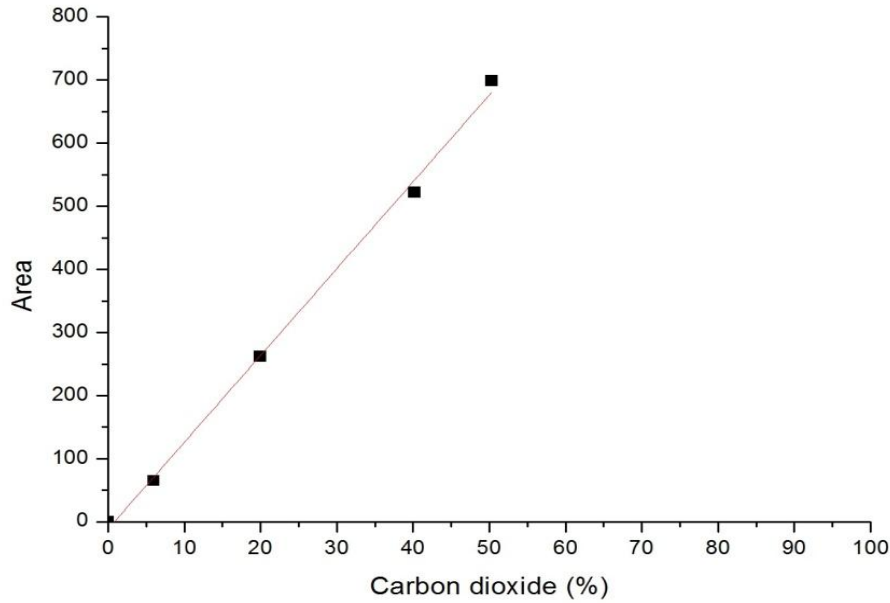
**Figure 4.5** Carbon dioxide utilization by *Chlorella vulgaris*

Maximum carbon fixation rates of *Chlorella vulgaris*, was calculated to be 586.50884 mg L<sup>-1</sup> day<sup>-1</sup>. When converted to per hour units this comes out to be 24.43787 mg L<sup>-1</sup> hour<sup>-1</sup>. This is well within the reported values by Jacob-Lopes *et al.* [43], by *Aphanothece microscopica Nageli*, which was between the ranges of 3.45 to 45.78 mg L<sup>-1</sup> hour<sup>-1</sup>. As depicted in the figure the CO<sub>2</sub> utilization rates tends to drop after day 4. This may be due to photo-inhibition of the lower layer of cells by the upper layer of cells. In earlier studies, performed by Sydney *et al.* using *Chlorella vulgaris*, better carbon dioxide fixation was obtained at 5% CO<sub>2</sub> [44].

#### 4.6 CO<sub>2</sub> bio-fixation ratios

Concentrations of inlet and outlet gas streams were monitored using a gas chromatograph. CO<sub>2</sub> bio-fixation was calculated using the two. Prior to this, the GC had to be calibrated using

pre-mixed volumetric ratios of N<sub>2</sub> and CO<sub>2</sub>. Various volumetric fractions of CO<sub>2</sub> used for calibration were 6%, 20%, 40.2%, 50.3%. The calibration curve is given in Figure 4.6.



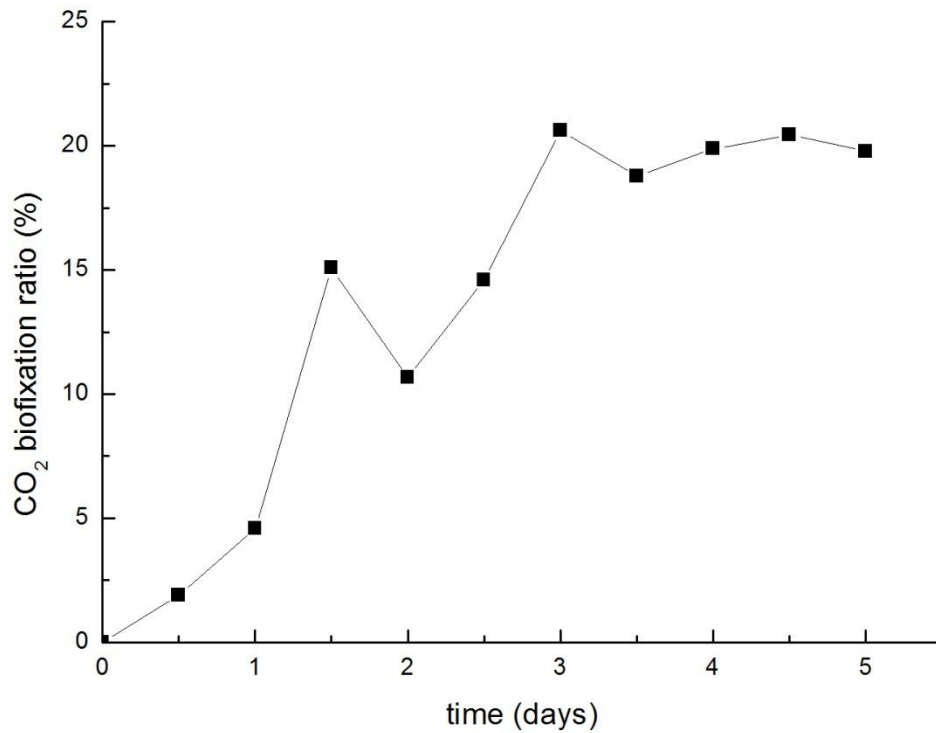
**Figure 4.6** GC calibration curve for CO<sub>2</sub>

The Y-axis representing area corresponds to the area of the electronic signals generated on injection of CO<sub>2</sub>. The equation for co-relating CO<sub>2</sub> percentage to volumetric fraction is as follows.

$$y=13.44968x-11.178343 ; R^2 = 0.99949$$

where y represents the area under the curve generated by electrical impulse and x represents the CO<sub>2</sub> percent by volume.

Maximum overall bio-fixation ratio was obtained was 20%. The bio-fixation ratios over the cultivation period are given in Figure 4.7.



**Figure 4.7** CO<sub>2</sub> bio-fixation ratios

The best fixation rates were seen between day 2 and day 3. This can be co-related with highest photosynthetic efficiency which was observed during that period. The data trend seems to relate well with the growth patterns. In both figures 4.6 and 4.7, the CO<sub>2</sub> fixation tends to stagnate or drop which indicates that the micro-algal strain has reached a stagnation phase in its' growth.

## CHAPTER -5 CONCLUSIONS AND FUTURE RECOMMENDATIONS

### 5.1 Conclusions

Micro-algae use carbon dioxide to facilitate photo-synthesis to store carbon in the form of starch and other intra-cellular constituents. *Chlorella vulgaris* has already been reported to fix CO<sub>2</sub> over time. The conclusions obtained from our studies can be summarised as follows-

- All algal cells produced are spherical and monodispersed.
- Effective hydrodynamic size of 2.37  $\mu\text{m}$
- Maximum specific growth rate was 0.596155  $\text{mg L}^{-1} \text{day}^{-1}$
- Maximum carbon fixation rates of *Chlorella vulgaris* 586.50884  $\text{mg L}^{-1} \text{day}^{-1}$
- At present cultivation conditions, cell activity is beginning to stagnate
- At lower inlet CO<sub>2</sub> fractions, higher CO<sub>2</sub> fixation maybe obtained

### 5.2 Future Recommendations

Additionally, *Chlorella* strains tend to produce higher concentration of fatty acids, as compared to other strains which indicate the high potential of *Chlorella vulgaris* to produce biodiesel. This is ideally produced at around 5% inlet CO<sub>2</sub> concentration. Further studies, which can be carried out, to investigate the potential of biofuel productivity can be carried out in the future. Elemental analysis as well as FAME (Fatty acid methyl ester) testing, will indicate their potential in this area. Other biochemical tests such as Lowry Bronsted assay for proteins and starch determination will lead to better understanding of the conversion of CO<sub>2</sub> into the intermediate forms.

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