

Chapter 2

CO₂ Sequestration Through Algal Biomass Production

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2.1 Introduction

The world is facing the threat of global warming. This is associated with the modernization and increasing dependency of human beings on the fossil fuel. Fossil fuel are necessary for providing the increasing demand on energy. However, their combustion produces greenhouse gases such as carbon dioxide, methane, ozone, NO_x, water vapour etc. (Kumar et al., 2011). CO₂ is also continuously being added into the earth's atmosphere through several natural sources such as volcanic eruptions, combustion of organic matters, autotrophic and heterotrophic respiration (Kumar and Das, 2014; Sharma et al., 2011). However, robust natural mechanisms of CO₂ capture could maintain the balance of CO₂ in the earth's atmosphere. Global carbon cycle is disturbed mainly due to anthropogenic emissions of CO₂ because of human activity (Kumar and Das, 2014). Coal is the major contributor of CO₂, which is in the range of 14–17 % depending upon its quality. Therefore, coal-based industries such as cement, steel and thermal power plants pollute the earth's environment to a greater extent.

The greenhouse gases trap the solar heat and prevent it from dissipating into the space. This increases the earth's temperature. The ecosystem of earth is affected by even a little increase in the global mean temperature. Melting glaciers, sea level rise, unpredicted rainfall and drought are some examples of global warming. Besides these, global warming increases the soil microbe's respiration, which further adds CO₂ into the earth's atmosphere (Bardgett et al., 2008). CO₂ is the major greenhouse gas of the flue gas (Kumar and Das, 2014). Keeling curve indicates a sharp rise in the global CO₂ concentration after 1950 (Kumar et al., 2011). In July, 2014, CO₂ concentration in the earth's atmosphere was 399.00 ppm as measured at Mauna Loa

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Observatory, Hawaii, USA (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>). This is significantly higher than the range of the CO₂ concentration (180–300 ppm) measured over the history of earth. Similarly, CO₂ concentration was found to increase at the rate of 2.05 ppm per year in 2013 compared to 0.94 ppm per year in 1959 (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>).

Thus, CO₂ sequestration is imperative to combat the challenges of global warming. Several natural processes continuously remove CO₂ from the atmosphere. For example, large mass of oceanic water dissolves atmospheric CO₂ and forms weak acids, which eventually forms bicarbonate due to reaction between carbonate anions and water (Kumar and Das, 2014; Falkowski et al., 2000). Similarly, large quantity of CO₂ is continuously sent to the bottom of the ocean due to differential solubility of CO₂ in cold and hot saline water. Besides these natural processes of CO₂ capture, CO₂ can be sequestered artificially, either by physical or biological methods. Physical methods involve natural mineral carbonation, scrubbing and geological (depleted oil and gas reservoirs) and oceanic injection (deep aquifers) etc. (Mirjafari et al., 2007; Allen et al., 2005). These methods are expensive as it also involves the collection, transportation and storage of CO₂ gas. In addition, it poses potential threat to the environment due to possibility of the accidental leakage of CO₂ (Bachu, 2000). Biological methods are another alternative, which mainly involves the photosynthesis process by oceanic phytoplanktons, terrestrial plants, algae and use of carbonic anhydrase (CA) enzyme. In addition, some non-photosynthetic bacteria also utilize CO₂ for their metabolic activity (Kumar and Das, 2014).

As an aggressive move, CO₂ sequestration through algal biomass production is a promising option. CO₂ sequestered algal biomass can be further utilized for the biofuel productions (biodiesel, bioethanol, biohydrogen) and several other value added products of industrial importance (Kumar et al., 2011; Loubiere et al., 2009). Microalgae can be cultivated in non-agriculture land or even in brown fields. Contrary to energy crops such as soybeans, algae can use various water sources ranging from wastewater to saline water. Furthermore, waste containing different volatile fatty acids can also be used for the supplementation of nutrient required for the algal growth. The photosynthetic efficiency of microalgae is nearly 10 to 50 times higher than that of terrestrial plants (Kumar and Das, 2014; Li et al., 2008). This results into higher specific growth rate and biomass productivity. Moreover, their cultivation is relatively easier and biomass can be easily managed during its processing for the extraction of value-added products. In addition, genetic modification targeting desired products is easy. Terrestrial plants are effective in sequestering CO₂ at low CO₂ concentration. Contrary to this, microalgae are efficient in growing at a relatively higher CO₂ concentration such as flue gas. Algal biomass can be directly used as food or food supplements as these are rich in protein, carbohydrates, essential fatty acids, antioxidants and several other biomolecules beneficial for the body. In fact, *Nostoc* sp. was used as food nearly 2000 years back during Chinese famine (Kumar and Das, 2014; Spolaore et al., 2006).

Thus, the present study aimed to summarize the different bottlenecks and the present state of art in CO₂ sequestration process through algal biomass production.

It also highlights the mechanisms of CO₂ sequestration process. The microalgal cultivation systems such as open ponds and photobioreactors are discussed in details.

2.2 Microbiology

Microalgae and cyanobacteria are distributed throughout the biosphere with an immense range of genetic diversity. Up until now, nearly 35,000 species have been described in the literature (Sydney et al., 2014). However, their actual number may be much higher. They can be found as unicells, colonies and extended filaments, growing even under extreme climatic conditions from aquatic to terrestrial places (Kumar et al., 2011). Their uniqueness is due to the presence of chlorophyll and their CO₂ fixing ability through photosynthesis in a single cell.

It is always desired to isolate a robust strain of algae, which can withstand the fluctuating physico-chemical conditions such as temperature, pH, light and CO₂ concentration and can grow efficiently in the open cultivation system. The low chances of contamination, the high CO₂ fixation rate and the product formation rate are the other desired characteristics of the algae. In the closed photobioreactor such as airlift photobioreactor, shear stress varies in different sections. Therefore, algal species having tolerance to high shear stress are other desired characteristics of the robust strain. Some of well known microalgal species used for the large scale production are described below.

Chlorella spp. are simple, non-motile and spherical unicellular eukaryotic green microalgae that have a thick cell wall (100–200 nm). *Chlorella* sp. measure between 2 and 10 μm. *Chlorella* sp. have a high concentration of chlorophyll and photosynthetic ability compared to higher plants. The optimal CO₂ concentration for the growth of *Chlorella* sp. was found in the range of 5–6 % (Kumar and Das, 2012; de Morais and Costa, 2007). They are known to have a high specific growth rate (μ): 0.08 h⁻¹ for *Chlorella vulgaris* and 0.11 h⁻¹ for *Chlorella sorokiniana* (Cordero et al., 2011). *Chlorella* sp. are known as an attractive source of food due to high concentration of protein (nearly 50 %) and essential nutrients. *Scenedesmus* spp. are freshwater, non-motile microalgae. Various biotic and abiotic factors determine the extent of aggregation of these microalgae. For example, they are unicellular in axenic condition and known to form colonies in xenic system of the natural system (Geng et al., 2014). *Botryococcus* spp. are found in colony held together within a lipid biofilm matrix. They are characterized by their slow growth rate and accumulation of the high amount of hydrocarbons (up to 50 %, w/w), which is released outside the cells.

Chlorococcum spp. are spherical unicellular marine green microalgae with cells of diameter about 10 μm. They have a high tolerance to CO₂ concentrations and can be grown to a very large cell density (Iwasaki et al., 1998). *Dunaliella* spp. are biflagellated, motile, unicellular marine green microalgae. They are mostly round in shape of diameter 9 to 11 μm. They have a high tolerance for salts, temperature and light (Segovia et al., 2003). *Dunaliella* lacks the cell walls, which make them an

extremely fragile microorganism. They reproduce by binary fission with no evidence of cell lysis, encystment or spore formation (Segovia et al., 2003). *Dunaliella* is well known for the production of wide varieties of commercial products such as β -carotene (up to 14 % w/w). β -carotene can be easily extracted due to the easy rupture of their cell wall. *Nannochloropsis* spp. are small, non-motile spherical shaped and mostly marine in nature. They have only chlorophyll-*a* and completely lack chlorophyll-*b* and *c*, which make them distinct from other related microalgae. *N. salina*, *N. Gaditana* and *N. oceanic* are some of the well known species of *Nannochloropsis*. Along with CO₂ sequestration, they have wide industrial applications. They are oleaginous strains, rich in polyunsaturated fatty acids and other value-added products such as astaxanthin, zeaxanthin and canthaxanthin (Lubian et al., 2000). In addition, they are choice of researchers because of easier genetic manipulation aimed for the genetic improvement. *Haematococcus* sp. are mobile, single-celled green algae (Chlorophyta), capable of synthesizing high amount of astaxanthin in response to environmental conditions, ranging from 1 to 5 % of dry cell weight (Wan et al., 2014). They have two stages of growth: growth stage (green cell) followed by astaxanthin accumulation stage (red cell). Therefore, *Haematococcus* cell changes its resistance to shear stress along the growth cycle. They have a slow growth rate (nearly 1.20 div d⁻¹), which make them prone to contamination (González-López et al., 2012).

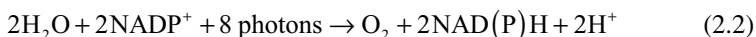
Cyanobacteria are Gram-negative bacteria having a thin peptidoglycan layer. Cyanobacteria can be single cellular or multicellular. Filamentous colonies of cyanobacteria can have different type of cells such as vegetative cells, akinetes and heterocysts, which have a uniqueness to carry out complete oxygenic photosynthesis, storing food reserves and nitrogen fixing ability, respectively (Kumar et al., 2011). These are the only prokaryotic microorganism having the ability to reduce both atmospheric CO₂ and nitrogen into carbohydrates and ammonia, respectively. Similar to microalgae and plants, cyanobacteria carry out oxygenic photosynthesis, involving both PSII and PSI. In anaerobic conditions, they use only PSI, causing anoxygenic photosynthesis. Nitrogen is fixed by the enzymes nitrogenase in the heterocysts of cyanobacteria. Carbohydrates and ammonia are utilized for the growth of cyanobacteria. *Synechocystis* is unicellular cyanobacteria. *Anabaena* and *Aphanothece* are linear multi-cellular, nitrogen fixing cyanobacteria. *Spirulina* sp. are freshwater, long, thin spiral thread like cyanobacteria, largely cultivated for the mass scale production. *Spirulina* has filaments (or trichomes) of helical shape in liquid medium and spiral shape in solid medium, which is the characteristics of the genus. Their large size (up to 1 mm in length and 12 μ m in diameter) makes them easier in harvesting. Chlorophyll and phycocyanin are their main photosynthetic pigments. *Spirulina* sp. usually grow efficiently at alkaline pH with optimal pH in the range of 8.5 to 11. They are known for the food or food supplements as *Spirulina* cells are rich in protein, polyunsaturated fatty acids (PUFAs), vitamins, antioxidants and several important minerals.

2.3 Mechanism of CO₂ Fixation

2.3.1 Photosynthetic Pathway

Photosynthesis is the process in which CO₂ is fixed into carbohydrates using the light energy and water. Algae use this process to prepare their food for growth and survival. Chloroplast is the site of photosynthesis in the microalgae. Whereas, in cyanobacteria, photosynthetic apparatus is found in the cytoplasm. It has two stages: light dependent and light independent. Chlorophyll and light harvesting complex harness the light energy and conserve it in the form of energy currencies such as ATP and NADPH formed in the light dependent stage. In the light independent stage, ATP and NADPH are consumed during the synthesis of carbohydrates. Photosynthetic apparatus consisting of protein complexes, electron carriers and lipid molecules are located in or around the thylakoid membranes. Thylakoid membrane has two reaction centers called PSII and PSI, connected with several electron carriers such as plastoquinone, cytochrome b₆f complex and plastocyanin (PC) in the order of increasing redox potential. This arrangement of electron carriers allows the flow of electron from negative redox potential to positive redox potential. PSII and PSI are specialized to absorb the light of wavelength 680 nm and 700 nm, respectively. Electron carriers are arranged in the order of increasing redox potential. The water split at PSII into protons, electrons and oxygen molecule with the help of light of 680 nm (Eq. 2.1). Protons generated by splitting of water are accumulated into the lumen of the thylakoid membrane and oxygen escapes from the cell. A proton gradient is generated across the thylakoid membranes due to accumulation of the protons in the lumen. These protons eventually escape from the lumen into the stroma generating ATP with the help of ATP synthase. On the other hand, electron gets excited at the PSII and travels to PSI by the linear electron transport chain. At PSI, it is further excited with the help of light of 700 nm. Antenna chlorophyll molecules help in harnessing the light at PSI. Excited electron reduces Ferredoxin (Fd), which is loosely bounded to the thylakoid membrane from outside (Kumar and Das, 2014). Reduced Fd transfers its electron to the ferredoxin NADP⁺ reductase (FNR), which catalyses the formation of NADPH using NADP⁺ and H⁺ coming from the lumen.

It should be noted that electron carriers transfer only one electron at a time with the help of one photon at each of the reaction center. Eventually the light energy is stored in the form of NAD(P)H and utilized in the light independent phase during CO₂ fixation using Calvin cycle. The production of two moles of NAD(P)H requires eight photons and two moles of water as shown in Eq. 2.2.



2.3.2 Calvin Cycle

CO_2 is reduced as carbohydrates into the algal cells during the light independent phase through the process called Calvin cycle. Calvin cycle takes place in the stroma of chloroplast and all the participating proteins are found residing outside the thylakoid membrane in the aqueous phase (Kumar and Das, 2014; Whitmarsh and Govindjee, 1999). Calvin cycle has broadly three steps: carboxylation, reduction and regeneration. Several complex reactions are involved in the Calvin cycle. However, only major reactions are shown in the diagram (Fig. 2.1).

Ribulose-1,5-bisphosphate (RuBP), a 5-carbon compound, is the starting compound of the Calvin cycle (Calvin and Benson, 1948). Ribulose 1,5-bisphosphate carboxylase (RuBisCO) catalyzed the carboxylation reaction between RuBP and CO_2 forming two molecules of glycerate 3-phosphate. In the reduction reaction, glycerate 3-phosphate is reduced to glyceraldehyde 3-phosphate (Eq. 2.3). It is followed by regeneration reaction, where five molecules of glyceraldehyde 3-phosphate is needed to regenerate one molecule of RuBP to start the Calvin cycle again and remaining one molecule is channeled for the synthesis of cellular biosynthetic materials for immediate energy source. Carbohydrates such as sucrose is transported to

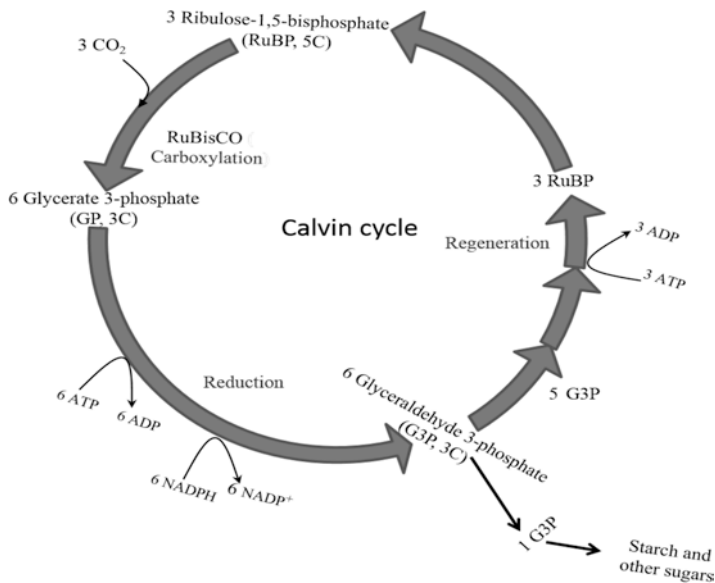
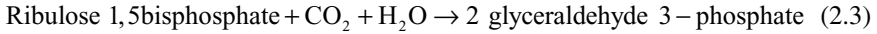
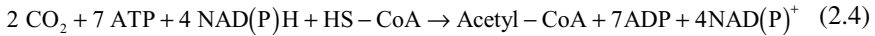


Fig. 2.1 Schematic diagram of the Calvin cycle. Only major intermediates have been shown. (Modified from Kumar and Das, 2014)

the cytosol, where these are stored in the form of glycogen and starch in cyanobacteria and green algae, respectively (Kumar and Das, 2014).



Calvin cycle is the most energy intensive processes among all the known pathways of CO₂ capture (Fast and Papoutsakis, 2012). It requires seven molecules of ATP and four molecules of NAD(P)H to transform two molecules of CO₂ into acetyl-CoA through the Calvin cycle as shown in Eq. 2.4 (Kumar and Das, 2014; Fast and Papoutsakis, 2012).



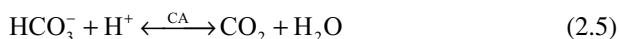
One reduced CO₂ molecule stores energy equivalent to 1400 kJ, which is produced at the expense of two moles of NAD(P)H. A synthesis of two moles of NAD(P)H in the light dependent phase requires eight moles of photons containing total energy of 3840 kJ mol⁻¹. In this way, maximum theoretical efficiency of the process can be calculated as 36.5 % (Kumar and Das, 2014).

2.3.3 CO₂ Concentrating Mechanisms

Cells of microalgae and cyanobacteria are simple in structure, due to which they cannot stop the diffusion of CO₂ to outside (Kumar and Das, 2014). They have low affinity for CO₂. In addition, CO₂ diffuses very slowly (nearly 10,000 times) in the aqueous solution compared to that in air (Kumar and Das, 2014). These factors limit the CO₂ availability to the RuBisCO to discharge carboxylase activity of fixing CO₂ into cellular components. In fact, under normal atmospheric condition, RuBisCO is only half saturated with the CO₂. Most of the algal cells have developed carbon concentrating mechanisms (CCMs) to cope with the low partial pressure of CO₂ outside the cell. The purpose of CCMs is to enhance the local partial pressure of CO₂ (up to 1000 times higher than the outside culture) near the RuBisCO (carboxysome in cyanobacteria and pyrenoid in microalgae). The affinity constant (K_m) of cyanobacteria and microalgae for CO₂ are generally ≥200 μM and ~20 μM, respectively. This may be the reason of need to store large amount of bicarbonate within the cells of cyanobacteria (100 fold) compared to microalgae (20 fold).

CO₂ is available inside the liquid culture in the form of gaseous dissolved CO₂ and different other chemical forms such as carbonic acids (H₂CO₃), bicarbonate (HCO₃⁻), carbonate (CO₃²⁻) depending upon the pH of the culture. Most of algal species were found to assimilate both CO₂ or HCO₃⁻. However, only HCO₃⁻ is delivered into the cytosol regardless of the chemical species assimilated by the whole cells. This may have several reasons. For example, intracellular pH of cytoplasm is

near 8, hence HCO_3^- predominant inside the cytoplasm. HCO_3^- , being a charged species, is unable to diffuse out the lipid bilayer of the cell membrane. CCMs have been extensively studied in the cyanobacteria. DIC is transported across the membranes by several mechanisms: transport of HCO_3^- into the cytosol by the ABC type transporter utilizing ATP, transport of HCO_3^- into the cytosol with the help of $\text{HCO}_3^-/\text{Na}^+$ symporter or Na^+/H^+ antiporter, transport of CO_2 by NADH dehydrogenase having constitutively low or inducible high affinity for CO_2 . Besides these, HCO_3^- can have direct access to the cytoplasm; however its transfer rate across the membrane does not contribute significantly in the DIC accumulation compared to its active transport (Kumar and Das, 2014). The HCO_3^- transported to cytosol is further delivered into the carboxysome/pyrenoids. The pyrenoid is a densely packed compartment in the chloroplast containing RuBisCO. Both the carboxysome and pyrenoids have limited permeability of CO_2 and act as a store house for CO_2 . Cyanobacteria have several carboxysomes (5 to 20 in number) into each cell depending upon species and growth conditions. RuBisCO enzymes have affinity only for gaseous CO_2 . Therefore, inorganic carbon must be converted back into gaseous CO_2 . Carbonic anhydrase (CA) does this job and catalyses the interconversion of inorganic carbon back to gaseous CO_2 as shown in Eq. 2.5. Under low partial pressure of CO_2 outside the algal cells, CA over-expresses to maintain high local CO_2 concentration. High concentration of local CO_2 activates the carboxylase activity of RuBisCO and fix the CO_2 more efficiently. CCMs have been less studied in the microalgae compared to cyanobacteria. Contrary to HCO_3^- in cyanobacteria, CO_2 is the predominant species entering into the microalgae such as *Chlamydomonas reinhardtii* (Spalding, 2008). The uptake of CO_2 into the whole cell and through the chloroplast of microalgae is due to diffusion and mediated transfer, respectively (Kumar and Das, 2014).



2.3.4 CO_2 Fixation Through Algal Biomass Production

The CO_2 is fixed during algal growth through several processes such as biomass formation, mineralization (transformation of gaseous CO_2 into chemical species such as bicarbonates, carbonates) and production of extracellular products such as polysaccharides, volatile organic compounds, organohalogenes, hormones etc. (Sydney et al., 2014). Among them, biomass formation is the major factor, which was found to account for 70–88 % of total CO_2 fixation during algal cultivation (Sydney et al., 2014). CO_2 fixed during other processes are wasted or easily lost to the environment. Therefore, most of the researchers quantify the CO_2 fixation taking only biomass formation into account. The CO_2 fixation ability of microalgae and cyanobacteria varies (Table 2.1).

Table 2.1 Biomass productivity and CO₂ fixation rate of different microalgae

Algal species	Photobioreactor (volume)	Biomass productivity (mg L ⁻¹ d ⁻¹)	CO ₂ fixation (mg L ⁻¹ d ⁻¹)	References
<i>Anabaena</i> sp. ATCC 33047	Bubble column	310	1450	Lopez et al., 2009
<i>Aphanothecemicroscopica</i>	Bubble column (3.2 L)	301	562	Jacob-Lopes et al., 2009
<i>Spirulina platensis</i>	BioFlowfermentor (11 L)	156	319	Sydney et al., 2010
<i>Spirulina</i> sp.	Vertical tubular (1.8 L)	280	–	de Morais and Costa, 2007
<i>Synechocystis aquaticlis</i>	Vertical flat plate (24 L)	78	128	Zhang et al., 2001
<i>Botryococcusbraunii</i>	BioFlowfermentor (11 L)	207	497	Sydney et al., 2010
	–	27	–	Yoo et al., 2010
<i>Chlorella vulgaris</i>	BioFlowfermentor (11 L)	129	252	Sydney et al., 2010
	–	298	624	Yun et al., 1997
	–	105	–	Yoo et al., 2010
<i>Chlorella sorokiniana</i>	Airlift reactor (1.4 L)	338	619	Kumar and Das, 2012
<i>Chlorococcum littorale</i>	Vertical tubular (20 L)	530	900	Kurano et al., 1995
	Vertical tubular (15 L)	120	200	Yang et al., 2013
<i>Scenedesmusobliquus</i>	–	218	–	Yoo et al., 2010
	Erlenmeyer flask (0.2 L)	142	253	Basu et al., 2013
	Vertical tubular (1.8 L)	160	–	de Morais and Costa, 2007
<i>Dunaliellatertiolecta</i>	BioFlowfermentor (11 L)	143	272	Sydney et al., 2010
<i>Nannochloropsisoculata</i>	Cylindrical (0.8 L)	480	902	Chiu et al., 2009

The elemental composition of biomass of algal species such as *Chlorella* sp. changes depending upon experimental conditions such as the concentration of CO₂ used for growing algae. C, H, N and S were reported in the range of 46.1 %–50.1 %, 6.1 %–7.74 %, 6.7 %–8.52 % and <0.5 % (w/w), respectively (Kumar et al., 2014; Rizzo et al., 2013; Friis et al., 1998). The molecular formula of the algal biomass of microalgae can be determined from the relative percent of C, H and N present in the algal biomass and can be represented in the form of CH_xN_yO_z. The corresponding molecular weight of algal biomass of *C. sorokiniana* was in the range of 24–26 g (Kumar et al., 2014). For example, 24.04 g per mole of biomass of *C. sorokiniana* was obtained, when the air was the sole carbon source. Material balance can be written in the form of Eq. 2.6 (Kumar et al., 2014).



Assuming 50 % carbon content in the algal cells, 1.83 kg of CO₂ from the air is fixed for the production of one kg of algal biomass along with the release of nearly 1.9 kg of oxygen (Kumar et al., 2014; Kumar and Das, 2012).

2.4 Bottlenecks in the Algal Biomass Production

2.4.1 Temperature

Temperature is one of the most important factors influencing all the biological systems including algae. Each of the microalgal species has an optimal temperature at which they grow most suitably. The temperature affects the cellular enzymatic reactions, including the cytosolic pH. The optimal temperature of most of the algal species varies in the range of 25–35 °C. Algal growth is more sensitive to high temperature compared to low temperature. In fact, a little increase over the optimum temperature may severely affect the algal growth. The low temperature reduces the cellular activities such as limiting the electron transport chain (ETC), activity of CA etc. (Kumar et al., 2014). The high temperature affects the CO₂ fixation due to inhibitory effects on the cellular physiology and denaturing of essential proteins/enzymes. For example, photosystem II is very thermolabile and prone to denature at higher temperature. Besides these, an increase in photorespiration, a decrease in RuBisCO affinity for CO₂ and a decrease in CO₂ to O₂ solubility ratio are the other vital phenomena negatively affecting the CO₂ fixation at higher temperature (Kumar et al., 2014; Kumar et al., 2011).

The target of most of the CO₂ capture using microalgae is from the flue gas, generally having higher temperature. In addition, outdoor cultivation of algae is influenced by the local climatic temperature, which is higher for the countries closer to the equator and lower for the countries located far away from the equator. Further, the temperature exposed to algal cells depends upon the time of light exposure and

the season variations. The use of thermophilic algae is an alternative for the fixation of CO₂ coming from the flue gas having high temperature.

2.4.2 pH

Most of the algae has neutral or slightly alkaline cytosolic pH as cellular enzymes are pH sensitive and become inactive at acidic conditions (Kumar et al., 2014). Therefore, the optimal pH of most of the algal species has been found in the range of 7 to 9. The extreme pH negatively affects the growth and CO₂ fixation by disrupting cellular processes. The introduction of CO₂ and other acidic gases such as SO_x and NO_x (generally present in the flue gas) in the culture medium decreases the pH. CO₂ dissolves in water by forming carbonic acids. The availability of carbonaceous species (aqueous CO₂, carbonic acids, bicarbonate and carbonate) to the algal cells depends upon the pH of the culture as the equilibrium shifts among them on change in the culture pH.

Along with the growth of the algae, pH generally increases. This may be due to consumption of CO₂ and other volatile fatty acids (if present in the medium). The rise in pH along the growth is higher at the inlet gas stream with low CO₂ concentration. This may be because of the release of hydroxide ions outside the cell at the expense of capture of H⁺ ions inside the thylakoid membranes during the activation of CCMs (Kumar and Das, 2012; Jacob-Lopes et al., 2008). The presence of nitrogen source in the medium also influences the pH of the culture broth. For example, the consumption of nitrate from the medium helps in the increase of alkalinity via OH⁻ production (Kumar et al., 2014; Hulatt and Thomas, 2011). Contrary to this, uptake of NH₄⁺ by the microalgae leads to H⁺ production (Goldman and Brewer, 1980).

2.4.3 Light

Algae fix the CO₂ in the presence of light during photosynthesis. Therefore, light is crucial in CO₂ capture through algal biomass production. Algal cells in the culture can experience broadly three different phases of light such as light limitation, light saturation and light inhibition (Kumar et al., 2013). The light utilization efficiency and biomass productivity proportionally increases with the light intensity till the cells become light saturated. The light saturation value of algae is in the range of 80–200 μmol photons m⁻² s⁻¹ (Kumar et al., 2014).

At low light intensity, photosynthesis becomes limiting. Lee and Pirt (1981) reported an increase in the cellular maintenance requirements during long time exposure of dark, which causes reduction in the biomass productivity. At high light intensity, photosynthesis is disrupted due to photoinhibition. The excess light energy is dissipated as fluorescence or heat through non-photochemical quenching (NPQ)

(Kumar et al., 2014; Yamakawa and Itoh, 2013). The photoinhibition disrupts the synthesis and degradation cycle (D1/D2 proteins) of light harvesting complex at PSII, eventually leading to inhibition of photosynthesis (Kumar et al., 2011). Along with the quantity of light, quality of light (light of a specific wavelength or light regime) also affects the algal biomass production. For example, in a study by Kim et al. (2014), the red light regime was found favoring the CO₂ fixation in *N. gaditana* followed by blue and white light regime. The pigmentation of algal cells exponentially reduces the light intensity inside the culture broth. The reduction of the size of the antenna molecules of algal cells (antenna size mutant) by molecular tools was found an effective way to decrease the pigment content and thus increasing the extent of light penetration inside the algal culture. This will eventually lead to better light energy utilization causing higher CO₂ fixation and biomass productivity. Proper mixing and efficient photobioreactor design are the other strategies for the effective distribution of light energy inside the algal culture and to improve the light utilization efficiency (Kumar et al., 2011).

2.4.4 *Mixing*

The importance of CO₂ for algal cultivation can be understood by the fact that the cost of the carbon accounts for 8–27 % of the total production cost of the algal biomass (Li et al., 2013). The bottlenecks of CO₂ transfer to the algal cells is due to higher resistance to the mass transfer of CO₂. RuBisCO is the crucial enzyme for the Calvin cycle used in CO₂ fixation. However, RuBisCO has low affinity for CO₂ compared with O₂ (Kumar and Das, 2013; Kumar et al., 2011). Moreover, CO₂ gas faces resistance to transfer from the gas phase to the liquid phase and from the liquid phase to inside the algal cells. Therefore, adequate supply of CO₂ in the culture is necessary. It has been estimated that CO₂ concentration greater than 65 μmol L⁻¹ at a pH of 8.5 is necessary for the optimal productivity of majority of microalgae (Weissman et al., 1988). However, the dissolved CO₂ concentration in water in equilibrium with air is nearly 10 μmol L⁻¹ at 25 °C.

2.4.5 *Substrate Inhibition*

An optimal CO₂ concentration in the inlet gas stream increases the specific growth rate and enhances the biomass productivity. The optimal CO₂ concentration was found in the range of 2 to 10 % in most of the microalgae. However, ability to tolerate CO₂ can be as high as 100 %. High CO₂ concentration causes substrate inhibition and environmental stress causing reduction in the algal growth. In addition to the environmental stress, high CO₂ concentration decreases the pH of the culture. Algae has higher CO₂ tolerance capability compared to terrestrial plants and therefore more effective in CO₂ sequestration. CO₂ tolerance ability of algal cells can be

enhanced by the method of adaptation in gradually higher CO₂ concentration. According to Miyairi (1995) drop in pH is less at higher temperature due to decrease in the solubility of CO₂ in the culture.

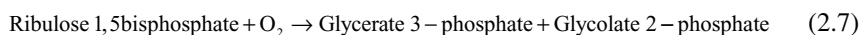
2.4.6 Product Inhibition

2.4.6.1 Biomass Concentration

Cell growth causes exponential decrease in the light penetration inside the photobioreactor due to self-shading effect by the microalgal cells. This is similar to the product inhibition. At fixed light intensity, algal cells may be in a light inhibition zone at low cell density and light limiting phase at higher cell density. The biomass concentration in the range of 1–2 g L⁻¹ was found optimum taking both biomass productivity and self-shading effect into consideration (Hu et al., 1996; Zhang et al., 2001). The continuous or semi-continuous method of algal cultivation may be an effective way to maintain optimum cell density.

2.4.6.2 Oxygen Accumulation

Accumulation of high concentration of oxygen leads to photooxidative damage to the algal cells. It has been reported that maximum tolerable dissolved oxygen concentration should not increase more than 400 % (>35 mg L⁻¹) of air saturation value (Kumar et al., 2011; Carvalho et al., 2006; Lee and Lee, 2003). RuBisCO enzyme has affinity for oxygen as well as carbon dioxide. In fact, affinity constant (K_m) for oxygenase activity is significantly lower than that of carboxylase activity. Therefore, RuBisCO participate in the CO₂ fixation only when the CO₂ concentration in the compartment is significantly higher. In case of oxygenase reaction, algal cells negatively affect the CO₂ fixation. For example, glycolate 2-phosphate is the end product when oxygenase dependent reaction of RuBisCO is active (Eq. 2.7). A significant amount of cellular energy is wasted during the formation of glycolate 2-phosphate, which is not required by algal cells. Further, glycolate 2-phosphate breaks down into glycine, which forms serine by combining with another glycine molecule resulting in a loss of CO₂ (Kumar and Das, 2014; Zeng et al., 2011). The loss of CO₂ may be up to 50 % of the algal biomass (Giordano et al., 2005). Furthermore, RuBP synthesis is affected because of loss of fixed carbon.



Shapes and sizes of the photobioreactor greatly influence the rise in dissolved oxygen (DO). For example, an increase in DO is a severe problem for the horizontal tubular photobioreactor but for the photobioreactor such as airlift, it is not a critical

issue due to having an open gas disengagement zone (Kumar and Das, 2014; Kumar et al., 2011; Lee and Lee, 2003).

2.5 Algal Biomass Cultivation System

2.5.1 Open Ponds

Open ponds have shallow configuration, which allow maximum light penetration inside the culture. Open ponds can be categorized into four types: shallow big ponds, tanks, circular ponds and raceway ponds (Rao et al., 2012). Among them, raceway pond is considered as an attractive cultivation system. This is because of ease of mixing and better biomass productivity. In fact, nearly 95 % of the total world algal biomass facility is based on the raceway ponds (Mendoza et al., 2013). The large scale production of microalgae was started by Oswald and Golueke (1960) in the 1950s in a raceway pond using *Chlorella* sp.

Algal cultivation in open ponds has several advantages such as ease in operational handling, practical way of scaling up microalgal production facility, low initial investment cost, free solar radiation, low energy requirement for mixing etc. (Jorquera et al., 2010; Mendoza et al., 2013). The product extracted from the algal biomass obtained from the open ponds are comparatively cheaper than the photobioreactor. For example, the cost of biodiesel obtained from raceway ponds was calculated as 12.73 USD per gallon, which was nearly 2.5 times lower to that of photobioreactor (Richardson et al., 2012). Open ponds also have several drawbacks: areal biomass productivity is in the range of 0.12–0.48 g L⁻¹ d⁻¹, which is nearly 2 to 8 times lower than the photobioreactor (Ketheesan and Nirmalakhandan, 2012; Brennan and Owende, 2010). Algal culture is susceptible to contamination, which decreases the biomass productivity and makes algal biomass unfit for the extraction of high value product. Only few selected algal species such as *Chlorella*, *Haematococcus*, *Dunaliella*, *Nannochloropsis*, *Spirulina*, etc. were found successful in growing in the open system. Further, the shallow depth of the open ponds allows low residence time to the introduced CO₂ gas, which in turn reduces CO₂ transformation efficiency into cellular constituents. In fact, nearly 80–90 % of the sparged gas is lost to the atmosphere (Richmond, 2004; Weissman et al., 1988). Besides these, open system has disadvantages in controlling the physico-chemical parameters such as availability of light, agitation, pH, temperature and nutrient concentrations (Kumar et al., 2011). The fluctuation in temperature and light availability during diurnal cycles and seasonal variations are other challenges with the open systems (Brennan and Owende, 2010).

Open ponds are generally light and carbon limited. The pond depth of the culture influence the light utilization efficiency, temperature variation in the culture, mixing (especially vertical mixing) and associated power consumption by the paddle-wheel. An increase in the pond depth enhances the total areal productivity of the algal biomass. For example, nearly 134–200 % increase in the areal productivity was obtained, when pond depth was 40 cm compared to 20 cm deep raceway ponds

(Sutherland et al., 2014). Similarly, mixing is very important for algal biomass productivity as it determines the nutrient and light availability to the algal cells. It has been found that optimum mixing enhances the biomass productivity by nearly 10 fold (Hreiz et al., 2014). The vertical mixing in the raceway ponds is more important as it limits the culture depth and hence the total areal biomass productivity. The vertical mixing causes cells to move from bottom (dark zone) to surface (light zone) of the open ponds. Therefore, it ensures the frequency by which each of the algal cells experience solar light exposure. Further, vertical mixing also prevents cells from either photolimitation or photoinhibition (Chiaromonti et al., 2013).

2.5.2 General Raceway Ponds in Operation

2.5.2.1 The Paddle-Wheel Driven Raceway Ponds

Paddle-wheels are mechanically simple to construct, require little maintenance and generate gentle mixing. These are traditionally used in the raceway ponds. In the raceway ponds, clearance zone between paddle wheel and the bottom floor is kept small (Chiaromonti et al., 2013). In this way, paddle-wheel acts as a positive displacement pump due to no backflow of the liquid. A wide recirculation flow of liquid can be visualized long after the bend of the raceway ponds showing the effectiveness of the raceway ponds (Chiaromonti et al., 2013). However, paddle-wheel driven raceway ponds have disadvantage of high power consumption and low vertical mixing (Xu et al., 2014). Paddle-wheel driven raceway pond in operation is shown in Fig. 2.2.



Fig. 2.2 Raceway pond for the large scale microalgae cultivation (200 m³) at HighTech Algae Center, Hadong, South Korea.

2.5.2.2 Sump-Assisted Raceway Ponds

Most of the design of the raceway ponds is focused on the enhancement of CO₂-liquid contact time. For example, a sump was incorporated in the raceway ponds and gas bubbles were introduced at the bottom of the sump (Mendoza et al., 2013). Sump is a long tubular dig, at the bottom of which gas bubbles are introduced. In this way, CO₂ bubbles have to diffuse out into the atmosphere by passing through a long water column. This increases the CO₂-liquid contact time and thus better CO₂ utilization efficiency and minimum CO₂ loss into the atmosphere (Craggs et al., 2012). Areal biomass productivity in the sump-assisted raceway pond was found 17 g m⁻² d⁻¹ with CO₂ capture of nearly 66 % from the inlet gas stream (deGodos et al. 2014).

2.5.2.3 Airlift/Split Sump-Assisted Raceway Ponds

The sump-assisted raceway pond was further modified by introducing baffles in the sump. Baffle partitioned the sump into two halves. In this way, this acts as an airlift reactor with one side as riser and the other side as downcomer. The CO₂-air gas mixture is introduced from the riser. The density difference between the two portions of the split sump was the driving force for the liquid movement in the raceway ponds. For further enhancing the CO₂-liquid contact time, normal air was used to circulate and generate culture velocity to the liquid and CO₂ was introduced in the counter-current direction at half the height of the downcomer (deGodos et al., 2014). The airlift driven raceway pond was compared with the traditional paddle-wheel driven raceway ponds. Ketheesan and Nirmalakhandan (2012) claimed the reduction of nearly 80 % energy consumption in airlift-driven raceway ponds at same culture velocity.

2.5.3 Photobioreactors

Microalgae are cultivated in photobioreactor to overcome the bottlenecks of the open ponds. Better control of process parameters such as pH, temperature, mixing, light penetration etc., maintaining axenic culture and necessity to obtain higher biomass productivity are some of the advantages of growing algae in the photobioreactor. Photobioreactors are the closed system, similar to bioreactors with additional provision of supply of light into the photobioreactor. This requires a high surface area to volume ratio (S/V ratio) and transparent surface of the photobioreactor. Necessity to consider these two criteria make photobioreactors difficult to scale-up. Photobioreactors are generally made up of transparent materials such as glass, plexiglass or polycarbonate (Kumar et al., 2011). Closed surface of the photobioreactor helps in introducing the gaseous CO₂ in a controlled way and with increased CO₂ gas-liquid contact time. Higher residence time of the CO₂ into the

photobioreactor enhances its mass transfer and correspondingly CO₂ sequestration efficiency and algal biomass productivity. Photobioreactors designed for CO₂ sequestration take advantage of using gaseous CO₂ for the supply of carbon source as well as ensuring sufficient mixing. However, in some photobioreactors such as stirred tank, agitation is provided by mechanical devices as well as sparging with CO₂-rich gas. It is always desirable to introduce the CO₂-rich gas mixture in order to reduce energy consumption. Mixing with CO₂ rich gas stream also helps in the removal of oxygen generated during the algal growth.

2.5.3.1 Vertical Tubular Photobioreactor

Vertical tubular photobioreactors are most commonly used for the CO₂ sequestration. Height to diameter ratio is generally kept greater than two (Kumar et al., 2011). The CO₂-rich gas stream is passed at the bottom of the cylindrical vessel through sparger. Long liquid column in the cylindrical vessel increases the CO₂-liquid contact time. Very tiny gas bubbles can be produced by using sparger with suitable pore size, which further enhances the volumetric mass transfer of gaseous CO₂. Advantages of vertical tubular photobioreactors are in low infrastructure cost, ease in the design and handle, absence of moving parts, relatively homogeneous culture environment, higher S/V ratio and efficient removal of unused gas mixture and photosynthetically produced O₂ (Kumar et al., 2011). Working volume of this type of reactor is generally 80 % of the total volume. Based on the placement of the light source, vertical tubular photobioreactors can be called as externally or internally illuminated photobioreactor.

Airlift and bubble column photobioreactors are the well known examples of vertical tubular photobioreactor. Airlift and bubble column are different from each other due to difference in mixing (Kumar and Das, 2014). Airlift photobioreactors have additionally draft tube placed at the center of the bubble column. In this way, airlift reactor has two different zones: one light zone (annular area) and another dark zone (central area). The CO₂-rich gas stream can be passed either through a central tube or through the annular space. The zone of airlift, where sparger is placed, is named as riser whereas the other one is called downcomer (Kumar and Das, 2013; Kumar and Das, 2014). Riser is also called gassed zone, where liquid has less density compared to downcomer. The density difference between the two sides drives the liquid culture in axial circular and oriented motion along the length of the photobioreactor. The direction of movement of the culture broth is from riser to downcomer. It is to be noted that maintaining the liquid level above the draft tube is necessary in order to experience the axial movement of liquid. Algal cells periodically move through the light and dark zone due to elliptical movement of the culture along the length of the reactor (Kumar and Das, 2013; Kumar et al., 2011). This gives flashing light effect to algal cells (Barbosa et al., 2003). The liquid moves in zig-zag and haphazardly on removing the draft tube (bubble column), leaving many dead zones in the photobioreactor. Vertical tubular photobioreactors have the disad-

vantages of poor control of temperature and low surface area for receiving the sunlight.

2.5.3.2 Horizontal Tubular Photobioreactor

Horizontal tubular reactors are long tubular tubes placed horizontally generally made up of transparent polypropylene acrylic or polyvinylchloride pipes with small internal diameter. The CO₂-rich gas stream is introduced from one side of the tube through the dedicated gas exchange unit. The longer length of pipes gives higher residence time to the CO₂ gas bubbles. This type of reactor is mostly used for algal cultivation in the outdoor. Parallel tubes are oriented in such a way to receive a maximum of solar light. Disadvantages of this type of photobioreactor are poor gas disengagement, wall growth and cleaning problem. This becomes more severe as the length of the tubes increases. Oxygen produced during the photosynthesis get trapped inside the photobioreactor. Lee and Lee (2003) observed an increase in photosynthetically produced DO concentration up to 400 % in horizontal tubular photobioreactor. Temperature is controlled by sparging the cold water over the surface of the tubes or overlapping tubes of cold water and photobioreactor or putting the light harvesting unit in the pool of cold water (Kumar and Das, 2014).

2.5.3.3 Helical Tubular Photobioreactor

Helical tubular photobioreactor is a flexible, transparent and a coiled shape structure placed vertically. It has separate gas engagement unit attached at the top of the coil. The CO₂-rich gas stream is passed from the bottom opening of the helical reactor. The long helical unit gives a high S/V ratio. In addition, CO₂ gas has high residence time inside the reactor, which increases the CO₂ sequestration efficiency. The light source can be placed co-axially at the center of the photobioreactor to minimize the loss of light energy and better biomass productivity. Helical photobioreactor can be scaled-up by increasing the number of coils in it. However, accumulation of oxygen increases proportionally with the increase in the coil of helical tubular photobioreactor (Kumar and Das, 2014). It has the advantage of requiring a small land footprint. Helical tubular photobioreactor suffers from many disadvantages such as fouling inside the tube, shear stress during the introducing of the gas, trapping of photosynthetically produced oxygen, most importantly design and handling of the photobioreactor. Helical reactors were further modified and transformed into a cone shape for better solar light utilization efficiency (Kumar and Das, 2014; Kumar et al., 2011; Watanabe and Hall, 1996).

2.5.3.4 Flat Panel Photobioreactor

The flat panel reactor has a cuboidal shape with flat surface at two sides for receiving the light. Most of the studies are focused on the flat panel photobioreactor. It has a high S/V ratio and better illuminated light path length. The agitation is carried out by the CO₂-rich gas stream, which is introduced from the bottom through tubes with fine holes. Some researchers placed the tubes upto half of the length of the photobioreactor to induce spiral mixing pattern. Flat panel photobioreactors have been further modified by putting draft tubes inside it. Similar to airlift photobioreactor, flat panel photobioreactor can have the riser and downcomer. The simple arrangement of flat plate reactor makes it easy to construct with any desired light path length. However, wall growth, difficulty in cleaning and low biomass productivity per unit space are some of the problems with this type of reactor. An increase in the hydrostatic pressure with the increase in the volume of the reactor during scale-up is another problem associated with this type of arrangement.

2.6 Conclusions

Large scale microalgal cultivation has potential to sequester significant amounts of CO₂ through biomass production and thus contributing in reducing the global warming effect. Several physico-chemical parameters influence the algal biomass productivity. Therefore, it is important to optimize these parameters and grow the microalgae at optimal environmental conditions. All the cultivation systems have their own merits and demerits. The raceway ponds are more suited for the cheap and large scale algal cultivation. Closed photobioreactor is used for growing the algae in controlled physico-chemical conditions, which will result into better biomass productivity. Various shapes and design of the closed photobioreactor have an influence on the algal biomass productivity. In addition, longer residence time of CO₂ in the closed photobioreactor can improve the CO₂ sequestration efficiency of microalgae. However, the cost effective biomass production in large scale is yet to achieve in the closed photobioreactor.

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