Chapter 12
Carbon Dioxide Sequestration by Biological Processes

Kanhaiya Kumar and Debabrata Das

12.1 Introduction

Carbon dioxide is one among many greenhouse gases such as methane, ozone, NO_x, and water vapor. Greenhouse gases act as blanket, which retain the outgoing sun's heat (infrared rays) into the earth's atmosphere. In absence of greenhouse gases, earth would be colder. Contrary to this, increase in any one of the greenhouse gases will result in extra trapping of heat, causing global warming. CO_2 constitutes very small portion of the gases present in the earth's atmosphere. Natural range of CO_2 concentration over the last 650,000 years was in the range of 180–300 ppmv. However, presently CO_2 concentration has crossed the natural range and reached as high as 397.34 ppmv in March 2013. Moreover, the Keeling curve reveals progressively faster rise in CO_2 concentration after industrialization [1]. Rate of increase in atmospheric CO_2 was 1.94 ppm/year in 2011, which was more than twice the estimated value in 1959 [1]. Temperature contributes significantly to make planet habitable for the living beings. Therefore, trace amount of CO_2 in the earth's atmosphere is required for maintaining stable temperature. Increase or decrease of few degrees of global temperature can have a devastating effect on earth. Global atmosphere and ocean temperature have increased by 0.6 °C and 0.3 °C, respectively, in the last century despite the fact that solar output witnessed decline in the same period [2]. Rise in temperature is the reason for climate change and melting of glaciers, thus causing rise in the sea level. In addition, global warming will cause increase in soil microbe's respiration and further addition of greenhouse gas CO_2 [3]. In the last century, global sea level rose by 17 cm [2]. Nearly 2 billion tons of CO_2 is being absorbed in the upper layer of ocean per year. Increase in CO_2 absorption resulted in the increase of acidification that adversely affects the aquatic life [2]. The International Panel on Climate Change

K. Kumar • D. Das (✉)
Department of Biotechnology, Indian Institute of Technology Kharagpur,
I.I.T. Kharagpur, India
e-mail: ddas.iitkgp@gmail.com

B.M. Bhanage and M. Arai (eds.), *Transformation and Utilization of Carbon Dioxide*, Green Chemistry and Sustainable Technology, DOI 10.1007/978-3-642-44988-8_12,

(IPCC) forecast increase in CO_2 concentration up to 570 ppmv by the end of the twenty-first century. This will cause nearly a rise of 1.9 °C of mean global temperature, causing an increase in mean sea level by 38 cm [4]. It is disputable to link rise in temperature with increase in CO_2 concentration. Some reports suggest present temperature rise is the result of natural cycle of rise and fall in temperature over the earth's history. However, some researchers believe present rise in temperature is due to an increase in CO_2 concentration. It becomes more important as some reports reveal unprecedented rate of increase in temperature is contrary to natural cycle of earth temperature observed so far [2].

CO_2 is being injected into the atmosphere from natural as well as artificial sources. Natural sources of CO_2 are volcanic eruptions, decomposition of organic matters, and autotrophic and heterotrophic respiration [5]. However, there is a natural mechanism of CO_2 removal such as oceanic and terrestrial CO_2 removal from the atmosphere. Imbalance in natural CO_2 addition and removal from the atmosphere is due to anthropogenic emission of CO_2 by human activity despite the fact that natural processes remove 50 % of the anthropogenic CO_2 emissions. Therefore, increasing consumption of fossil fuels is the main matter of concern due to the anthropogenic CO_2 emissions. Total annual anthropogenic CO_2 emission due to fossil fuel consumption is 29 Gt per year [6]. Today, power plants, cement, steel manufacturing industries, transportation, and household usages are dependent on fossil fuels [7]. Coal-fired power plants consume nearly one third of the total fossil fuel consumption and the remaining from fossil fuel usages in other sectors such as transportation, industry, and homes [2, 8]. Increase in human population and modernization are the reasons for the booming of these sectors.

In this scenario, it is necessary to adopt some strategies to sequester CO_2 from the earth's atmosphere. CO_2 sequestration strategies can be divided into abiotic and biotic categories. Abiotic categories involve scrubbing, mineral carbonation, and geological and ocean injection. Several techniques are available to separate CO_2 from the flue gas. Further, they can be stored at various locations such as oceans, deep aquifers, and depleted oil and gas reservoirs [9]. However, these methods can pose potential threat for safety and environmental impact due to accidental leakage especially in long term [10]. Biotic categories include CO_2 sequestration in oceanic, terrestrial, and secondary carbonates. There are several natural phenomena occurring in the ocean, which ultimately remove atmospheric CO_2 [11]. Oceanic CO_2 at the surface is in equilibrium with atmospheric CO_2. CO_2 dissolved in water forms weak acid, which reacts with carbonate anions and water to form bicarbonate. Continuous supplies of cations are required to maintain the buffering capacity of bicarbonate system. CO_2 solubility gradient is another phenomenon by which large amount of CO_2 is being sent to the bottom of ocean. CO_2 is more soluble in cold and saline water. Therefore, cold dense water masses at higher latitude especially at the North Atlantic and Southern Ocean confluence sink into the interior of ocean carrying huge amount of CO_2 [11]. This eventually gets trapped by less dense water at the top for several hundred years. Further, oceanic phytoplanktons play a vital role by absorbing atmospheric CO_2 at the surface of the ocean during photosynthesis. Nearly 25 % of carbon fixed by these processes sinks to the bottom of the

ocean. It has been estimated that 11–16 Gt of carbon is removed from the atmosphere by this process [11]. Oceanic phytoplanktonic photosynthesis lowers the atmospheric CO_2 by 150–200 ppmv [11]. CO_2 is also absorbed naturally such as through sedimentation. CO_2 conversion into bicarbonate rocks is a natural process. Nearly 2 Gt of carbon is sequestered in the soil through burial of decomposed organic material from plants, animals, biomass, and agriculture by microbes [6]. However, most of those are released back into the atmosphere during soil erosion and oxidation. Biochar is another promising material helpful in CO_2 removal. Biochar is charcoal, which is produced when smoldering biomass is burnt in limited oxygen producing little heat. The advantage of biochar in CO_2 sequestration is that it gives stability to carbon present in the biomass against microbe's oxidation [6] and, thus, prevents the release of CO_2 into the atmosphere.

Land-based plants consume CO_2 in photosynthesis and release CO_2 during respiration. However, there is net CO_2 sequestration from the atmosphere. Plants are not capable of utilizing the concentrated CO_2 present in flue gas. It has been estimated that afforesting 22 % of earth's terrestrial surface, which is equal to present forested land, is required to sequester CO_2 emitted due to fossil fuel usages [12]. Other reports say only 345 million hectares is available for afforestation, which will result in 1.5 Gt C annually [6]. This can lead to little less than 20 % reduction in anthropogenic CO_2 emission. However, afforestation in total available area is not possible due to huge pressure on land for other purposes. Each year nearly 10 % of total atmospheric CO_2 is fixed into carbohydrates by the photosynthetic process [13]. Total global CO_2 removal by land-based plants and oceanic phytoplanktons are 403 Gt CO_2 (equivalent to 110 Gt carbon) and 385 Gt CO_2, respectively [8]. Microalgae, seaweeds, and higher aquatic plants are other alternatives for CO_2 sequestration because of their photosynthetic ability. However, the latter two are not promising because of low CO_2 and other nutrient mass transfer [14]. They require high amount of energy input for mixing. However, seaweeds are more promising compared to higher aquatic plants because it is difficult to achieve proper water exchange at the submerged plants and their dense stands [14]. Therefore, seaweeds are grown near the seashore and shallow ocean systems. Carbon is provided by seawater exchange system but requires high amount of energy for water pumping.

Use of microalgae, cyanobacteria, and other bacteria is another alternative available for CO_2 sequestration. A large number of microbes are known capable of utilizing CO_2. Except microalgae, all require some inorganic reducing agent such as H_2, H_2S, NH_3, etc. [14]. Photosynthetic efficiency of algae is 10 times higher than that of terrestrial plants. They are efficient in growing at relative higher concentration of CO_2 compared to plants. This makes them a suitable candidate for CO_2 sequestration from the flue gas. Cultivation of algae requires less water, and they can be grown in non-fertile land. Further, their biomass can be used for food and feed supplements, for the production of biofuels such as biohydrogen, bioethanol, biodiesel, and industrially important biomolecules and biofertilizers [7, 15, 16].

Use of carbonic anhydrase (CA) is another thrust area for CO_2 sequestration. Carbon content of earth's lithosphere is 42 % w/w in the form of $CaCO_3$ and other

carbonates which indicates transformation of gaseous CO_2 into solid carbonates is a geological stable process and has potential to exploit for CO_2 sequestration [17]. One of the reactions, hydration of CO_2 to carbonic acids, is the rate limiting step in CO_2 mineralization reaction [18]. CA is the catalyst of this reaction, and the use of this enzyme has been found to enhance the rate of reaction manifold [19]. Advantages of using CA for CO_2 sequestration are eco-friendliness, cost-effectiveness, simple process, and abundance of CA enzymes among microorganisms [20].

Thus, the present study aims to summarize various biological processes such as use of plants, microalgae, cyanobacteria, and other non-photosynthetic microbes for CO_2 sequestration. It also attempts to highlight different pathways occurring in biological systems along with the use of CA for this cause.

12.2 Biological Processes of CO_2 Sequestration

12.2.1 CO_2 Sequestration by Plants

The amount of CO_2 required for the terrestrial plants and crops is very less, nearly equal to atmospheric CO_2 concentration. Limited number of reports are available, demonstrating the long-term effect of CO_2 concentration on plants because of high amount of time and space required along with variation in experimental conditions. Therefore, it is not clear that the rise in CO_2 concentration will increase the CO_2 sequestration in existing forests [21]. Most suitable CO_2 concentration for plants obtained in greenhouses was nearly three times (0.1 %) the atmospheric CO_2. An experiment conducted by Norby et al. (2005) by analyzing four Free Air Carbon dioxide Enrichment (FACE) studies on forest reported that there will be 23 % increase in the plant productivity at the predicted level of CO_2 in 2050 compared to that of the present atmospheric CO_2 concentration [22]. However, they cautioned that increment in productivity may not increase the long-term CO_2 sequestration. Most of the trees in forest (~95 % of all higher plants) are C3 type having positive photosynthetic response to elevated CO_2 concentration [21, 23, 24]. Nearly one third of the global land is covered by forest. In another report, on average, 60 % and 27 % enhancement of photosynthesis and growth, respectively, for trees have been reported [25]. Elevated CO_2 concentration decreases the stomata aperture opening and reduces the water loss. Trees planted at CO_2 spring in Italy were found growing at similar rate compared to that of normal atmospheric CO_2 concentration [26]. Respiration rate has been found to decline in elevated CO_2 environment with little improvement in the photosynthetic ability of the plants. Thus, it is impractical to use terrestrial plants and crops for CO_2 sequestration from highly rich CO_2 stream of flue gas emitted from power plants [14]. The effect of temperature, one of the associated parameters with CO_2 concentration, has also been studied. Net CO_2 removal rate by plants was found to increase with temperature [27]. Further increase in temperature from optimum resulted in decreased CO_2 sequestration

rate. Therefore, CO_2 sequestration rate by green plants varies from day to day and season to season. It was observed that in CO_2-enriched atmosphere, plants shift the optimum temperature of CO_2 sequestration to higher level. Moreover, at this condition, process was found to be less sensitive to increase in temperature [27]. Despite the fact that plants have limited applicability in CO_2 sequestration at highly CO_2-rich environment, afforestation has a number of advantages. Firstly, nearly 30–40 % of captured carbon from atmosphere gets stored in depth of soil system through plant roots [28]. Secondly, cultivated agricultural crops contribute large amount of atmospheric CO_2 removal (in the order of 190 t ha^{-1} C) into the soil system acting as perpetual sink [28]. Thirdly, the lack of proper nitrogen fertilizer for land-based plants is one of the limiting factors. Therefore, CO_2 removal rate by plants can be enhanced significantly by the use of nitrogen fertilizer [8].

12.2.1.1 Mechanism of CO_2 Fixation

Each leaf cell contains nearly 50 chloroplasts [13]. Stomata are the entry sites of CO_2 into the leaf where it interacts with the RuBisCO present in chloroplasts and gaseous CO_2 reduced into carbohydrates. Stomata of leaves have been found unresponsive to higher CO_2 concentration. RuBisCO is the key enzyme of the Calvin cycle for all the three types of the plant C3, C4, and CAM. K_m of CO_2 for the C3 plants is 15–25 μM, and carboxylase activity of RuBisCO operates below the K_m of CO_2 and is not more than 25–30 % (equilibrium concentration of CO_2 in water and air is nearly 10 μM) of its maximum capacity (24, 29). However, C3 plants do not concentrate CO_2 using carbon concentrating mechanisms (CCMs) probably because of having large amount of RuBisCO and the presence of highly active β type of CA in the thylakoid stroma (Table 12.1). RuBisCO has both oxygenase and carboxylase activity. K_m for oxygenase activity is 700 times lower than that of carboxylase activity. Therefore, increase in atmospheric CO_2 concentration is associated with not only the increase in CO_2 assimilation but also the reduction in photorespiration consuming significant amount of oxygen [24]. However, at higher CO_2 concentration, rate of photosynthesis is limited by the regeneration of the ribulose-1,5-bisphosphate (RuBP). Triose phosphate produced during Calvin cycle releases inorganic phosphate P_i which is essential for ATP synthesis and RuBP regeneration from phosphorylated intermediates. Therefore, under these circumstances, photosynthesis is called P_i or triose phosphate limited use limited [24]. Fixed CO_2 can have three fates: (a) some of the CO_2 releases back into the atmosphere through respiration; (b) some of the CO_2 transfers to the soil through root exudates, root death, litter fall, and coarse woody debris which after decomposition releases back the CO_2 into the atmosphere; and (c) some of the CO_2 gets stored in the wood [21]. Contrary to C3 plants, C4 plants have the ability to grow efficiently in stress condition such as high temperature, low water availability, high irradiance, and saline soils. C4 plants on land have thick-ended walls of bundle-sheath cells, which help them in preventing the leakage of CO_2 outside [30]. CCM activates in plants by spatial separation of CO_2 fixation by

Table 12.1 Comparative study of CCMs occurring in plants (C3 and C4), microalgae, and cyanobacteria [29, 47]

Type of photosynthesis	CCM efficiency in DIC acquisition	K_m (CO_2) of RuBisCO	HCO_3^- accumulation over ambient concentration	DIC preference	Location of CA	Carbon concentrating mechanisms	RuBisCO location
Plants (C3)	Least efficient	15–25 μM	N.A	CO_2 as substrate	Highly active CA in stroma of chloroplast	One carboxylation reaction to form C3 compound	Chloroplast stroma of most of the leaf cells
Plants (C4)	Intermediately efficient	28–60 μM	N.A	PEP carboxylase (affinity with HCO_3^- only) more abundant than C3 plants	Abundance of CA in the cytosol of mesophyll cells Absent in bundle-sheath cells	Two carboxylation reactions to form C4 compound. Carboxylation and decarboxylation reactions are spatially separated	Bundle-sheath cells
Microalgae	Less efficient than cyanobacteria	20 μM	20 fold	CO_2 is preferred. Both CO_2 and HCO_3^- inside the cytosol	Periplasmic space; cytosol, thylakoid lumen; pyrenoid	HCO_3^-/Na^+ symporter; Na^+/H^+ antiporter; NADHdh-induced transport; ATP-fueled HCO_3^- transport; CO_2 transporter	Pyrenoid inside chloroplast
Cyanobacteria	Most efficient	≥200 μM	100 fold	Preference is less specific. Only HCO_3^- inside cytosol	Carboxysomes	Direct transfer of HCO_3^-; CA-mediated CO_2 diffusion	Carboxysomes

Calvin cycle. Normally PEP carboxylase catalyzes the CO_2 fixation in the mesophyll cells. C4 acids transported to bundle-sheath cells are decomposed to gaseous CO_2 by the action of NADP-malic enzyme and other decarboxylases. Both these processes are temporarily separated in CAM plants. Detailed discussions on Calvin cycle and CCMs in C4 and CAM plants are discussed in another section.

12.2.1.2 Oceanic Fertilization

Oceanic fertilization can be defined by adopting a practice of supplementing limiting nutrients to the phytoplanktons, causing increase in photosynthetic efficiency and CO_2 removal rate. For example, micronutrient Fe was added in 10 × 10 km patches of ocean, and 30 times increase in chlorophyll concentration with increase in 100,000 kg of carbon fixation was found [4]. However, its practice should be adopted cautiously as it can negatively interfere with the marine ecosystem. In addition, sinking organic matters on decomposition may produce other stronger greenhouse gases such as methane and NO_x [31].

12.2.1.3 Forest Fertilization

The study conducted by Oren et al. (2001) revealed that the enriched CO_2 concentration (550 ppmv) had only marginal positive effect on the biomass carbon increment. However, synergistic gain was observed when plants were grown in enriched CO_2 as well as nutrient condition. The gain was threefold and twofold larger at the poor site and at the moderate site, respectively. Fertilizing at higher CO_2 concentration is more prominent than the ambient CO_2 concentration [32]. Further, application of fertilizer in paddy soils has been found favoring the growth of autotrophic microorganisms, resulting in increased CO_2 sequestration [33].

12.2.2 CO_2 Sequestration by Microalgae and Cyanobacteria

12.2.2.1 Microalgae

Photosynthesis in microalgae takes place in chloroplast. Apart from other pigments such as β-carotene and xanthophylls, they have chlorophyll a and chlorophyll b as the major pigments, which give them bright green color. Light requirement of the typical algae is lower than the higher plants [34]. Light conversion efficiency and productivity are proportional to the increase in light intensity till it attains saturation light intensity [35]. Saturation light intensity of algae such as *Chlorella* and *Scenedesmus* sp. is of order 200 μ mol m^{-2} s^{-1} [36]. Photosynthesis is coupled with release of O_2 as by-product. High dissolved oxygen (DO) in the culture (>35 mg L^{-1}) has inhibitory effect on the photosynthesis [7, 37]. In the closed

photobioreactor, DO level has been found to increase as high as 400 %, thus severally affecting the photosynthetic process [38]. Further, cations dissolved in medium also negatively affect the photosynthetic process. Algae carry net negative charge in their surface and hence are a potential adsorber of polyvalent cations present in the surrounding medium. Adsorption of polyvalent cations on the surface of algae causes morphological changes in the cell morphology or can replace/block the prosthetic metal atoms in the active site of relevant enzymes leading to photosynthesis inhibition [34].

12.2.2.2 Cyanobacteria

Cyanobacteria is a gram-negative bacteria. Compared to other gram-negative bacteria, the cell wall of cyanobacteria has thicker peptidoglycan layer [39]. Cyanobacteria lack organelles in the cell. Respiratory chain is located in thylakoid membrane and plasma membrane. Photosynthesis takes place in thylakoid located in the cytoplasm, and photosynthetic electron transport takes place in its membranes. The photosynthetic apparatus of cyanobacteria is similar to the chloroplast of green algae and plants. However, major difference is in the antenna system. Cyanobacteria lack chlorophyll b and depend primarily on chlorophyll a and specialized protein complex known as phycobilisomes for harvesting light energy. Light harvesting complex can be lipophilic as well as hydrophilic in nature. Lipophilic pigments such as chlorophyll a and carotenoids are located within the thylakoid membranes whereas hydrophilic antenna pigments are housed in phycobilisomes attached outside of thylakoid membranes. Examples of hydrophilic pigments are allophycocyanin (APC), phycocyanin (PC), phycoerythrin (PE), or phycoerythrocyanin (PEC) [40]. Similar to green algae and plants, cyanobacteria carry out oxygenic photosynthesis, releasing O_2. However, in anaerobic condition, they can also carry out anoxygenic photosynthesis using PSI. Sources of electrons are smaller organic compounds such as succinate, hydrogen sulfide, and thiosulfate instead of water [41]. Anoxygenic photosynthesis results in generation of ATP by cyclic photophosphorylation around the PSI.

12.2.2.3 Photosynthesis: Key for CO_2 Sequestration

Photosynthesis, the key biological process occurring in plants and a wide number of microorganisms such as algae and cyanobacteria, helps in mitigating atmospheric CO_2. Photosynthesis occurs in the chloroplasts of plants and algae. Chloroplasts contain the thylakoid vesicles arranged in stacks, containing photosynthetic apparatus. Contrary to plants and algae, prokaryotic cyanobacteria do not have fixed organelles for keeping photosynthetic apparatus. They are located in cytoplasm as free and isolated photosynthetic lamellae [42]. Biological system uses photosynthesis for harnessing solar energy for the preparation of food with the help of CO_2 and water. Photosynthesis consists of two distinct processes: light-dependent process and light-independent

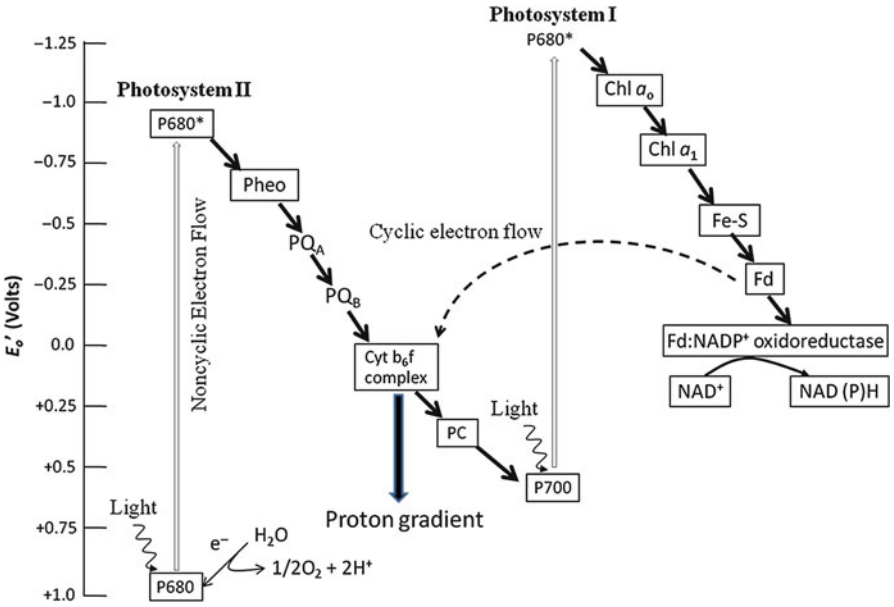

Fig. 12.1 Schematic diagram of Z scheme of photosynthetic process involving PSII and PSI. *Solid and dashed lines* show the noncyclic and cyclic flow of electrons. Abbreviation: PQ_A plastoquinone, PQ_B second quinine, *Chl* a_o chlorophyll a, *Chl* a_1 phylloquinone, *PC* plastocyanin [53]

process. In light-dependent process, they conserve light energy in the form of ATP and NADPH with the help of chlorophyll and light harvesting complex. Light-independent process utilizes ATP and NADPH for the fixation of CO_2 into triose phosphates, starch, sucrose, and other derived products. Photosynthetic apparatus of plants, algae, and cyanobacteria are similar in nature. Photosynthetic apparatus are found in the thylakoid membranes consisting of protein complex, electron carriers, and lipid molecules. The electron carriers are arranged in the shape of 90° tilted English alphabet Z. Two reaction centers, PSII and PSI, help in exciting the electrons by absorbing the light of 680 nm and 700 nm, respectively (Fig. 12.1). Two reaction centers are connected with series of electron carriers such as plastoquinone, cytochrome b_6f complex, and plastocyanine arranged in fixed increasing order of redox potential. Electron carriers between two centers help in transferring excited electrons at PSII to pass to PSI smoothly. Electrons at PSII come from breaking of water molecules into oxygen molecules, protons, and electrons. Two moles of water dissociates into four moles of protons and electrons and one mole of oxygen molecule (Eq. 12.1) [42].

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \tag{12.1}$$

Protons accumulate into the lumen of thylakoid membranes, generating proton gradient across the membrane. Concentration gradient drives protons outside thylakoid membrane through ATP synthase producing ATP. Thus, ATP synthase

produces not only energy for the cell but also avails protons for the reduction of $NADP^+$ to produce NADPH. At PSII, light energy causes charge separation between P680 and pheophytin, creating P680+/Pheo−. Pheophytin transfers the electrons to a permanently bounded molecule (QA) to photosystem II. At QA site plastoquinone receives single electron instead of two. QB and QA sites differ from each other as the former requires two electrons to reduce instead of one, which is the case of the latter, causing two turnovers for the complete reduction of plastoquinone at QB. Due to close proximity of QB site, protons added to plastoquinone during its reduction come from the outside aqueous phase of the membrane [13]. At photosystem I, most of the antenna chlorophyll molecules are attached to the reaction center proteins [13]. Plastoquinone then transfers their electrons to next electron carrier cytochrome b_6f complex with simultaneous release of protons into the lumen. Cytochrome b_6f complex is attached with membrane-bound protein complex. Plastocyanin (PC) acts as a last protein carrier delivering electrons to PSI. PSI again excites the electrons received from electron carriers. Here, excited electrons are used to reduce ferredoxin (Fd), a protein loosely attached with thylakoid membranes from outside. Reduced Fd interacts with ferredoxin $NADP^+$ reductase (FNR) and the latter catalyzes the reduction of $NADP^+$ to NADPH as shown in (Eq. 12.2).

$$2Fd_{red} + 2H^+ + NADP^+ \rightarrow 2Fd_{ox} + NAD(P)H + H^+ \quad (12.2)$$

Each of the photosystem contributes one photon to the transfer of one electron. Therefore, two photons are required for the transfer of one electron along the electron carriers to the $NADP^+$. Two moles of water and eight photons are required to produce two moles of NAD(P)H as shown in (Eq. 12.3).

$$2H_2O + 2\ NADP^+ + 8\ photons \rightarrow O_2 + 2NAD(P)H + 2H^+ \quad (12.3)$$

Reduction of one molecule of CO_2 requires two moles of NAD(P)H. Energy content in photon is 480 KJ/mol, whereas energy gathered after reduction of one reduced CO_2 molecule is equivalent to 1,400 KJ. Therefore, theoretical maximum efficiency of the process is 36.4 %.

12.2.2.4 Carbon Concentrating Mechanisms (CCMs)

Cyanobacteria and algae behave like C3 plants but have much less affinity for CO_2 [29]. However, they develop CCMs similar to C4 and CAM plants, causing better photosynthetic efficiency compared to C3 plants [30]. Their RuBisCO have oxygenase as well as carboxylase activities, depending upon the concentration of local CO_2 in the compartment housing RuBisCO. Carboxylase activity of RuBisCO activates under high CO_2 pressure, whereas low concentration of CO_2 forces it to undergo oxygenase activity. Oxygenase reaction has many disadvantages. Firstly, glycolate 2-phosphate is the end product of oxygenase activity of RuBisCO

(Eq. 12.4). It has no use to algal cells; therefore, significant amount of cellular energy is wasted by consuming it. Secondly, it releases previously fixed CO_2, during the carboxylase activity of RuBisCO, which causes the loss of nearly 50 % of algal biomass [43]. Metabolism of glycolate 2-phosphate produces glycine, which on condensing with another glycine molecule produces serine, resulting in the loss of CO_2 [44]. Thirdly, loss of fixed carbon further negatively interferes with the regeneration of RuBP, required for the smooth functioning of the cycle.

$$\text{Ribulose 1,5 bisphosphate} + O_2 \rightarrow \text{glycerate 3-phosphate} + \text{glycolate 2-phosphate} \quad (12.4)$$

Affinity constant (Km) of microalgae and cyanobacteria for CO_2 is high compared to C3 plants, indicating low affinity of RuBisCO for CO_2 (Table 12.1). Under normal atmospheric air, RuBisCO is only half-saturated with CO_2 [7, 29]. The diffusion of CO_2 in aqueous solution is 10,000 times slower than the CO_2 diffusion in air [30]. Poor diffusion of atmospheric CO_2 in aqueous solution and low affinity of CO_2 by algae, cyanobacteria, and some chemoautotrophic bacteria pose a limitation to the carboxylase activity of RuBisCO [45]. Therefore, for maintaining this, it is necessary to saturate the compartment containing RuBisCO with CO_2. Fortunately, algae have carbon concentrating mechanisms (CCMs), which help them to increase the local CO_2 even when there is lower CO_2 concentration outside the algal cells [46]. Contrary to plants, algae and cyanobacteria have single-cell CO_2 concentrating mechanisms. However, they have internal compartments within the chloroplasts (pyrenoid in algae and carboxysome in cyanobacteria). Increase in concentration of CO_2 at close proximity of RuBisCO has many advantages. Firstly, it activates RuBisCO. Secondly, it increases carboxylase activity of RuBisCO, and, thirdly, dissolved inorganic carbon (DIC) influx may help in maintaining internal pH and dissipating excess light energy [47]. CCMs can be divided broadly into two categories: biochemical and biophysical CO_2 pumps. CCMs help in accumulation of large number of intracellular inorganic carbon inside the RuBisCO compartment. However, inorganic carbons are not available for fixation by RuBisCO. They must be converted back into gaseous CO_2 for the action of RuBisCO. CA is a vital enzyme, which overexpresses under low external CO_2 environment. CA acts as a catalyst in the interconversion of inorganic carbon back to gaseous CO_2.

Biochemical CO_2 Pump

C4 Mechanism – Biochemical pumps are mostly found in the terrestrial plants. However, some phytoplanktons and macroalgae also have evidence of biochemical pumps. C4 mechanisms have also been found in macroalgae such as *Udotea flabellum* and *Thalassiosira weissflogii* [43]. The role of C4 mechanism is to biochemically transport the DIC from the site excessive in it to the site where RuBisCO is active [43]. As the name indicates, CO_2 is stored in a four-carbon

compound, oxaloacetate. Phosphoenolpyruvate (PEP) is the carrier molecule, which combines with HCO_3^- using PEP carboxylase to form oxaloacetate. PEP carboxylase utilizes bicarbonates rather than CO_2; therefore, the gaseous CO_2 entering into the mesophyll cell must be rapidly converted to bicarbonate with the help of carbonic anhydrase [48]. At physiological CO_2 levels and pH, Km (HCO_3^-) of PEP carboxylase and HCO_3^- concentration in the cytoplasm of mesophyll cells were estimated to be about 8 μM and 50 μM, respectively. Therefore, unlike RuBisCO, PEP carboxylase is always saturated with HCO_3^- at ambient CO_2 concentration. Therefore, CA is mostly confined to mesophyll cell in the C4 plants compared to chloroplast in C3 plants [49]. Oxaloacetate further readily reduced to malate by the action of NADP-malate dehydrogenase. In plants, malate is transported to bundle-sheath cell where it is decarboxylated by the action of NADP-malic enzyme. At the site of RuBisCO, malate can be decarboxylated to pyruvate and releases gaseous CO_2 for the action of RuBisCO enzyme in Calvin cycle.

CAM Mechanism – CAM plants are usually found in desert area. In cool night, guard cells open to receive CO_2, while in daytime, it is closed to prevent water loss. This mechanism is also proposed in brown macroalgae for the assimilation of photosynthetic inorganic carbon [43]. PEP comes from the starch accumulated during daytime using Calvin cycle. Enzymes and compounds taking part in CAM mechanism are similar to C4 mechanism. However, end storage compound malate is temporarily separated over time rather than spatially as in C4 plants [43]. PEP carboxylase catalyzes the reaction of PEP and HCO_3^- to form oxaloacetate. NADP-malic dehydrogenase reduces oxaloacetate to malate, which is transported to vacuole having low pH at night. In daytime, whole pathways get reversed back and malic acid transported back to cytosol for the decarboxylation reaction, flooding cytosol with CO_2. Guard cell is closed during the daytime, preventing CO_2 diffusion outside the cell. It is to be noted that PEP carboxylase activity is also under control to prevent wasteful synthesis of C4 compounds during daytime.

Biophysical CO_2 Pump

CCMs in Cyanobacteria

Being simple in structure, algae and cyanobacteria cannot stop the diffusion of CO_2 [30]. Carboxysome is the key for the success of CCMs in cyanobacteria. It acts as a storehouse for CO_2 having limited permeability for CO_2 leakage. The ultimate target of CCMs is to transport DIC to the storehouse, carboxysome. Along with cyanobacteria, carboxysomes are also found in some chemoautotrophic bacteria growing in CO_2 concentration lower than the K_m of RuBisCO [45]. Carboxysomes have mainly icosahedral structures with the diameter of 100–200 nm. The number of carboxysomes present in per cell of cyanobacteria varies from 5 to 20 depending upon the growth conditions and species to species [45].

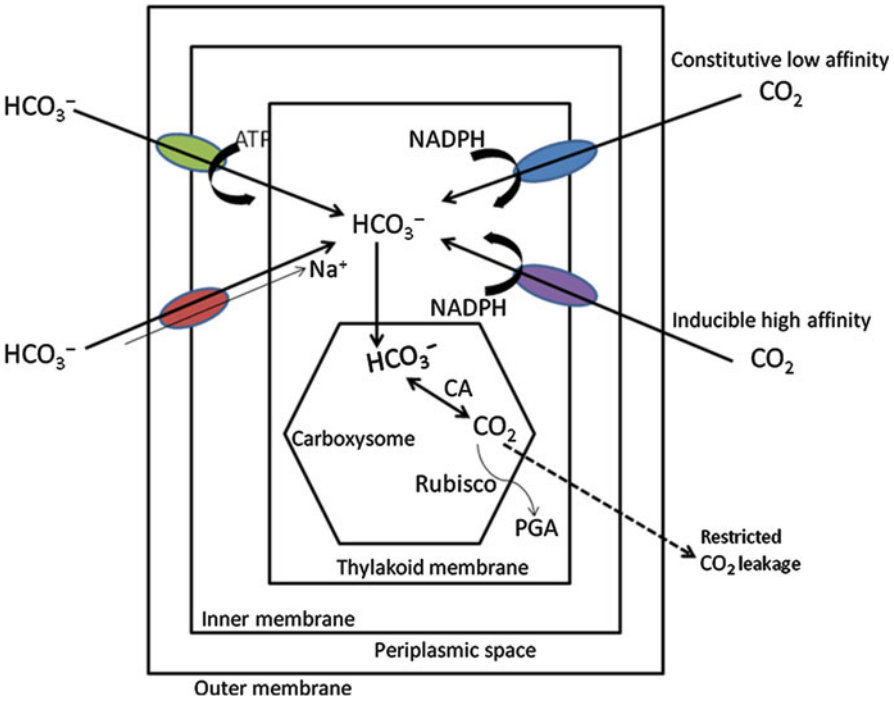

Fig. 12.2 Schematic diagram of CCMs occurring in cyanobacteria [29, 43, 49]

Most of algae and cyanobacteria examined so far have been found to have transporter for both CO_2 and HCO_3^-. However, few algae can also assimilate either CO_2 or HCO_3^-. CCMs are extensively studied in cyanobacteria compared to microalgae. Active transport occurs across the membrane having low permeability to DIC. In cyanobacteria, transport of CO_2 or HCO_3^- occurs via plasmalemma or thylakoid membranes. All the DIC removed from the outside by various DIC transporters are delivered only in the form of HCO_3^- into the cytosol of cell [45]. Bicarbonate concentrating ability of cyanobacteria is nearly five times greater than the microalgae, causing higher photosynthetic and CO_2 consumption efficiency of the former [30]. Outside, DIC including CO_2 penetrate the plasma membrane and reach the closest membrane of the chloroplast with the help of various transporter proteins. At least three mechanisms of active transport have been proposed in cyanobacteria (Fig. 12.2) [29, 49, 50]: (1) HCO_3^- can be transported to cytosol with the help of ABC-type transporter utilizing energy in the form of ATP, which is a high-affinity, low-CO_2-induced, and sodium-independent mode of HCO_3^- assimilation; (2) HCO_3^- transport into cytosol may also be the result of HCO_3^-/Na^+ symporter or the regulation of pH through Na^+/H^+ antiporter; and (3) for the active transport of CO_2, NADH dehydrogenase may have constitutively low or inducible high affinity for CO_2. In either case, CO_2 is first converted to HCO_3^- by NADHdh at plasmalemma of cyanobacteria and then

transported into the cytosol. Intracellular pH of cyanobacteria is near to 8, causing HCO_3^- as predominant species inside the cytoplasm (equilibrium ratio of HCO_3^- and CO_2 is near to 100). HCO_3^- is the charged ion and therefore cannot escape the lipid bilayer of the cyanobacteria [45]. HCO_3^- then diffuses into carboxysome where it is acted upon by CA enzyme to flood the compartment with gaseous CO_2 for the action of RuBisCO of Calvin cycle [43]. Remaining amount of CO_2 in carboxysome is diffused outward [47]. In addition, it has also been proposed to have direct access of HCO_3^- to cytoplasm of cyanobacteria. Rate of direct transport of HCO_3^- across plasma membrane is less significant compared to its active transport [51]. In yet another mechanism, compartment containing high concentration of HCO_3^- is acidified using proton pump and flooding with CO_2 (Eq. 12.5). In acidic compartment, HCO_3^- decomposes into CO_2 by the action of CA or proton-driven catalysis of HCO_3^- to CO_2. The high level of CO_2 then diffuses into the more alkaline compartment housing RuBisCO for the action of RuBisCO [43].

$$\text{Alkaline } (HCO_3^-) \rightarrow \text{ Acidic } (HCO_3^- \rightarrow CO_2) \rightarrow \text{ Alkaline } (CO_2) \quad (12.5)$$

CCMs in Algae

Compared to cyanobacteria, CCMs in microalgae are less understood because of more compartments inside the cell and very diverse group of microorganisms [29]. Microalgae composed of one or few cells do not have impermeable cell walls like plants to prevent leakage of CO_2. The challenging task is to prevent the leakage of concentrated CO_2 while allowing other nutrients to come in. The efficiency of acquisition of DIC depends on environmental conditions. For example, acquisition of DIC in low atmospheric CO_2 was found higher compared to high atmospheric CO_2 condition. However, the amount of RuBisCO did not change during adaptation from low to high CO_2 condition. This indicated the existence of transport system for the uptake of DIC into the cells. Cyanobacteria and microalgae can accumulate nearly 100- and 20-fold increase in HCO_3^- within the cells, respectively, over ambient CO_2 level [29]. DIC accumulation in algae is generally lower than cyanobacteria probably due to higher affinity of RuBisCO present in the former for CO_2. CCMs in microalgae have been hypothesized similar in nature as cyanobacteria by most of the researchers. Similar to carboxysome of cyanobacteria, microalgae also have a compartment (pyrenoid) in chloroplast densely packed with RuBisCO. Accumulation of charged HCO_3^- ions lessens the chance of leakage. Freshwater and marine green algae are capable of utilizing HCO_3^-. However, in most of the microalgae, CO_2 is main form of carbon entering into the cell and HCO_3^- in chloroplast. In *Chlamydomonas reinhardtii*, active transport of CO_2 has been found to have preference over HCO_3^- [51]. CO_2 uptake in whole cell is due to diffusion, while through the chloroplast, it is mediated by transfer [47]. A range of CAs participate in each of the compartment to maintain the equilibrium between CO_2 and HCO_3^- (Fig. 12.3). Eventually DIC entering into the pyrenoid is in the form of HCO_3^-, which needs to be converted back to CO_2 to enrich RuBisCO compartment for the carboxylase reaction. Cell membrane of pyrenoid does not

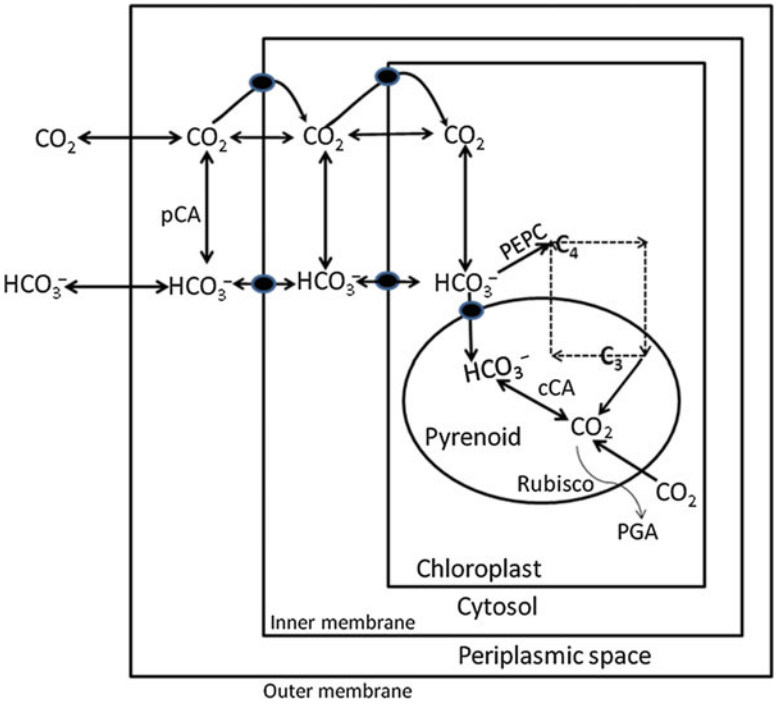

Fig. 12.3 Schematic diagram of CCMs occurring in green algae [29, 43, 49]

allow CO_2 to leak out, allowing sufficient time for RuBisCO to use CO_2 in the Calvin cycle. CA is an important enzyme for the successful operation of CCM. CA in microalgae is of different type and located in different places inside the cell. It has been found in periplasmic space, cytosol, as well as inside the pyrenoid. Direct uptake of HCO_3^- and CO_2 diffusion facilitated by periplasmic CA across the plasma membrane have been proposed [29]. Periplasmic CA probably helps in the diffusion of CO_2 and supply of HCO_3^- into the cytosol across the plasma membrane. However, in every case examined so far, electrochemical potential gradient for HCO_3^- was outward. By examining the HCO_3^- transport in *Ulva*, HCO_3^- uptake in microalgae has been proposed to occur through the HCO_3^-/OH^- antiport [47].

12.2.3 Bacteria

The non-photosynthetic bacteria play an important role in global carbon cycle. Advantages of these microorganisms are their ability to adapt to and survive extreme conditions of the environment. However, consumption of large amount of H_2 as electron donor limits their practical application [52]. Enzymes involved in

the CO_2 fixation pathway are sensitive to oxygen; therefore, they grow best in anaerobic environment [52]. Their ability to sequester CO_2 under anaerobic environment can be helpful for their application in O_2-deficient but CO_2-rich environment such as under the soil and flue gas [52].

Photosystem of some of the bacteria looks like either PSI or PSII. Lack of PSII deprives bacteria to use electrons of water and evolve oxygen as by-product of photosynthesis. Some simple inorganic or organic molecules substitute the water for the electrons needed to reduce CO_2 into useful simple sugar. They have bacteriochlorophyll, a family of molecules similar to chlorophyll, but absorb light in the range of 700–1,100 nm. Similar to oxygenic photosynthesis, electron transfer results in the generation of proton gradient across the thylakoid membrane. Outward protein gradient drives the proton out through ATP synthase, causing synthesis of ATP. Energy for CO_2 reduction comes from ATP and NADH. Electron carriers are quinone such as ubiquinone, menaquinone, and the cytochrome bc complex, which work similarly to cytochrome b_6f complex present in the oxygenic photosynthetic system [13].

Green gliding bacteria such as *Chloroflexus aurantiacus* harvest light using chlorosomes similar to green sulfur bacteria. CO_2 fixation in these microorganisms does not involve Calvin or Krebs cycle. They usually do photosynthesis under anaerobic condition. Green and purple bacterial membranes are in the form of lamellae, vesicles, or specialized structures (chlorosomes) where light reaction takes place. Sulfur purple bacteria such as *Chromatium vinosum* fix CO_2 for their survival using Calvin cycle.

12.2.3.1 Purple Bacteria

Photosynthetic machinery of purple bacteria such as *Rhodospirillum rubrum, Rhodopseudomonas viridis*, and *Rhodobacter sphaeroides* is of pheophytin-quinone type. Pheophytin is similar to chlorophyll but lacks central Mg^{2+} ions. Purple bacteria have single reaction center called P870. Electrons at the reaction center get excited, absorbing light of 870 nm. Excited electrons pass to cytochrome bc1 complex through pheophytin and quinine sequentially [53, 54]. Cytochrome bc1 complex is the hub that pumps the protons to generate proton gradient and electrons back to reaction center via cytochrome c_2 (Fig. 12.4a). Light-driven cyclic flow of electrons enables to produce ATP using ATP synthase. Purple bacteria are of two types: non-sulfur purple, such as *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*, and sulfur purple bacteria, such as *Chromatium vinosum*. Electron source for non-sulfur bacteria is organic compounds such as malate and succinate, whereas sulfur purple bacteria extract electrons from inorganic sulfur compounds such as hydrogen sulfide. CO_2 is fixed in purple bacteria using Calvin cycle.

12.2.3.2 Green Sulfur Bacteria

Reaction center of green sulfur bacteria such as *Chlorobium vibrioforme* and *Chlorobium thiosulfatophilum* is similar to PSI of oxygenic photosynthesis.

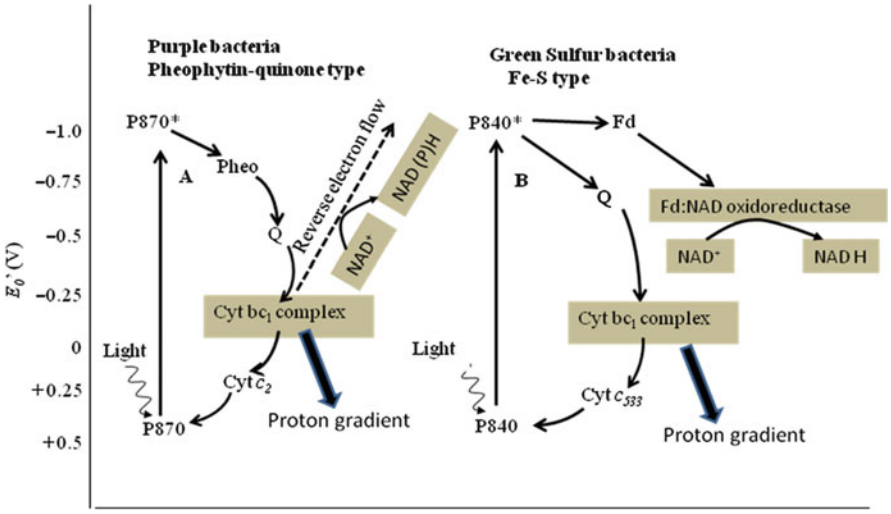

Fig. 12.4 Schematic diagram of photosynthetic process involving only PSI in (**a**) purple bacteria and (**b**) green sulfur bacteria. *Solid and dashed lines* show the noncyclic and cyclic flow of electrons. Abbreviation: *Q* quinine [53]

Similar to phycobilisomes of cyanobacteria, the antenna system of the green sulfur bacteria such as bacteriochlorophyll and carotenoids is contained in complexes known as chlorosomes attached to the surface of the photosynthetic membrane through baseplate containing antenna bacteriochlorophyll a [13]. Reaction center involved in green sulfur bacteria is called Fe–S type. Therefore, green sulfur bacteria are not dependent on the reverse electron flow for the carbon reduction as the reduced ferredoxin uses its electrons to reduce $NAD^+/NADP^+$ using ferredoxin–NAD(P)+ oxidoreductase enzyme. At reaction center, electrons get excited by absorbing light intensity of 840 nm. Excited electrons can follow two pathways: one cyclic flow of electrons back to the reaction center via Q, Cyt bc_1 complex, and Cyt c_{553}, and another noncyclic flow of electrons through iron–sulfur protein ferredoxin (Fd) to reduce NAD^+ to NADPH using ferredoxin/NAD reductase (Fig. 12.4b) [53, 54]. Similar to purple bacteria, protons are pumped by the cytochrome bc1 complex to generate ATP. Electrons at reaction center are replaced by the oxidation of H_2S to elemental S^o and then to SO_4^{2-}. Electron carriers in green sulfur bacteria are much better placed according to their electronegativity than the purple bacteria which ensure reduction of NAD without the need of reverse electron flow. Green sulfur bacteria reduce free CO_2 by reversing original tricarboxylic acid cycle (Krebs cycle) with the input of energy. Thus, green sulfur bacteria can fix CO_2 even in the absence of RuBisCO. Green sulfur bacteria extract both electrons and hydrogen from sulfur compounds [13].

12.3 CO_2 Sequestration Pathways

12.3.1 Calvin Cycle

CO_2 is fixed into carbohydrate in light-independent stage using Calvin–Benson cycle. Proteins participating in CO_2 fixation have been found outside the thylakoid membrane in aqueous phase [13]. CO_2 fixation reaction is catalyzed by the carboxylase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [55]. Calvin cycle can be divided into carboxylation, reduction, and regeneration reaction (Fig. 12.5). In carboxylation reaction, three molecules of CO_2 combine with three molecules of ribulose-1,5-bisphosphate (5 C) using carboxylase activity of RuBisCO enzyme to form six molecules of glycerate 3-phosphate (Eq. 12.6). Glycerate 3-phosphate further reduces to glyceraldehyde 3-phosphate in the reduction step. Reduction reaction is followed by regeneration reaction where ribulose-1,5-bisphosphate, the starting material of Calvin cycle, is regenerated using five molecules of glyceraldehyde 3-phosphate, while the remaining one molecule of glyceraldehyde 3-phosphate is used for the synthesizing biosynthetic material [56].

$$\text{Ribulose 1,5 bisphosphate} + CO_2 + H_2O \rightarrow 2\,\text{glyceraldehyde 3-phosphate} \quad (12.6)$$

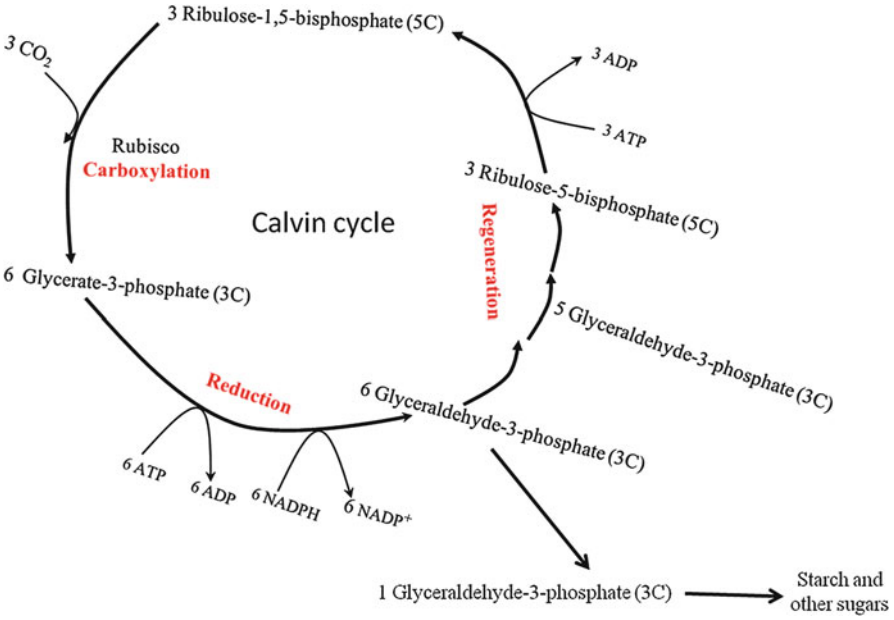

Fig. 12.5 Schematic diagram of Calvin cycle

Standard free energy for the synthesis of one mole of glucose is equal to 2,870 KJ [13]. Photosynthetic process is further generalized by Van Niel (Eq. 12.7).

$$CO_2 + 2H_2A + \text{light} \rightarrow (CH_2O)_n + 2A + H_2O \tag{12.7}$$

where A is O and S for oxygenic photosynthesis and anoxygenic photosynthesis (taking H_2S as electron donor), respectively. Later, it was demonstrated by Van Niel that molecular oxygen comes from dissociation of water rather than CO_2. The end product of Calvin cycle, glyceraldehyde 3-phosphate, is used in synthesizing cellular biosynthetic material for immediate energy source and sucrose that is transported to cytosol for storage as starch in chloroplast of green algae and glycogen in cyanobacteria [57]. Considering acetyl-CoA as the end product, seven molecules of ATP and four molecules of NAD(P)H are required to reduce two molecules of CO_2. Thus, Calvin cycle is the most energy intensive pathway for CO_2 sequestration [58].

$$2\,CO_2 + 7\,ATP + 4\,NAD(P)H + HS\text{-}CoA \rightarrow \text{Acetyl-CoA} + 7\,ADP + 4\,NAD\,(P)^+ \tag{12.8}$$

12.3.2 Wood–Ljungdahl (WL) or Reductive Acetyl-CoA Pathway

Wood–Ljungdahl (WL) or reductive acetyl-CoA pathway is a bidirectional pathway, which is predominant primarily in strict anaerobic bacteria and archaea of phyla Firmicutes and Euryarchaeota, respectively [58]. This pathway is used for chemoautotrophic carbon fixation in acetogens such as *Clostridium thermoaceticum* and *Acetobacterium woodii* and methanogens such as *Methanobacterium thermoautotrophicum* and most autotrophic sulfate reducers such as *Desulfobacterium autotrophicum*. Wood–Ljungdahl (WL) pathway was found to be the most efficient non-photosynthetic pathway based on the most expensive substrate (i.e., H_2 or electrons), for the production of acetate and ethanol [58]. Reductive acetyl-CoA pathway uses only four moles of H_2 to form one mole of acetate. Two molecules of CO_2 directly combine to form one molecule of acetate. This pathway is different from other six known CO_2 fixation pathways as it does not undergo in cyclic manner. In addition to the use of Fd for reduction reaction, carbon monoxide dehydrogenase and acetyl-CoA synthase are the main enzymes involved in this pathway. These enzymes are very sensitive to oxygen. It has both carbonyl as well as methyl components (Fig. 12.6). Out of two molecules of CO_2, one is reduced to carbonyl group (C=O) catalyzed by CO dehydrogenase, and the other CO_2 is captured on special tetrahydrofolate cofactor and reduced to a methyl group. Carbonyl group bonded with enzyme is combined with the methyl group to form acetyl-CoA by enzyme acetyl-CoA synthase complex. The reducing equivalents for the pathway are obtained by oxidation of molecular hydrogen during autotrophic growth or NADH and reduced ferredoxin during heterotrophic growth [59]. For the formation of one molecule of acetyl-CoA using two molecules

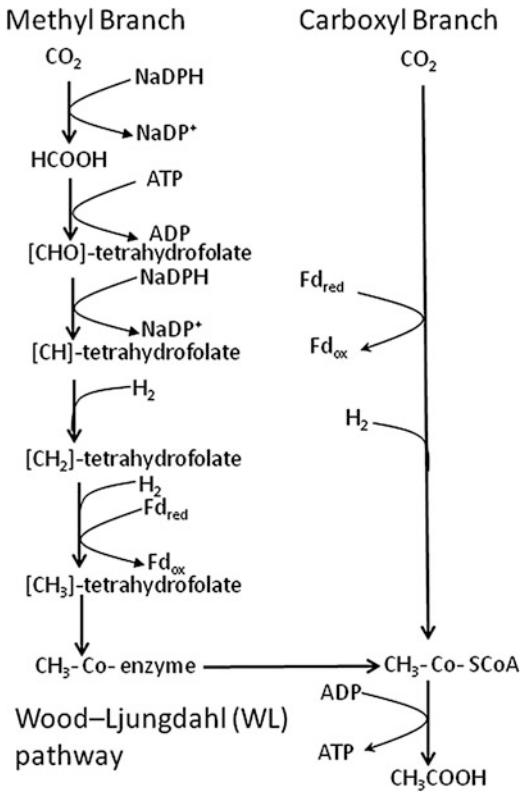

Fig. 12.6 Schematic diagram of Wood–Ljungdahl (WL) or reductive acetyl-CoA pathway. (Reprinted from Ref. [60]. Copyright 2011, with permission from Elsevier)

of CO_2, WL pathways need one molecule of ATP and four molecules of NAD(P)H (Eq. 12.9) [58]. Among the four pathways discussed above, WL pathway is most efficient based on CO_2 sequestration per ATP consumed. Moreover, ATP consumed in WL pathway is even less than one, as some of the energy is conserved in the membrane gradient in the form of ATP. rTCA cycle comes next to WL pathway in terms of energy efficiency. Both the pathways are found in highly reduced environments in which carbon reduction is more favorable [58].

$$2\,CO_2 + 1\,ATP + 4\,NAD(P)H + HS\text{-}CoA \rightarrow \text{Acetyl-CoA} + 1\,ADP + 4\,NAD(P)^+ \quad (12.9)$$

12.3.3 Reductive Tricarboxylic Acid Cycle (rTCA) or Reverse Citric Acid Cycle

Reductive tricarboxylic acid cycle (rTCA) has been found in bacteria under anaerobic or microaerobic conditions. Bacteria dwelling under these conditions are green sulfur bacteria such as *Chlorobium* sp. (*Chlorobium limicola, Chlorobium*

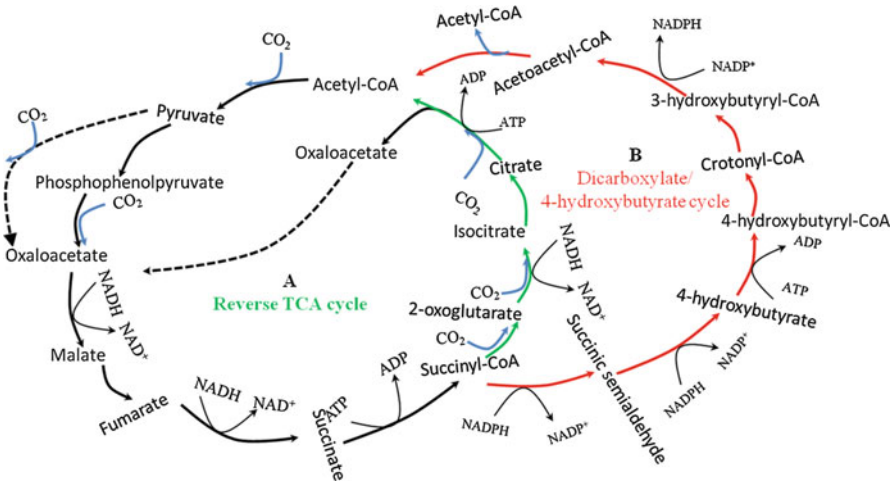

Fig. 12.7 Schematic diagram of (**a**) reductive tricarboxylic acid cycle (rTCA) or reverse citric acid cycle shown in *green arrow* and (**b**) dicarboxylate/4-hydroxybutyrate cycle shown in *red arrow*. *Black arrows* showing reaction pathways are common to both of them (Reprinted from Ref. [60]. Copyright 2011, with permission from Elsevier)

thiosulfatophilum, etc.), sulfur-reducing bacteria (*Desulfobacter*), knallgas bacteria or hydrogen-oxidizing bacteria (*Hydrogenobacter, Aquifex*), and archaea (*Thermoproteus*). They utilize this cycle to fix atmospheric CO_2 through anoxygenic photosynthesis [59]. Reductive citric acid cycle is oxidative citric acid cycle running in reverse direction [61]. In TCA cycle, one molecule of acetyl-CoA breaks down to form two molecules of CO_2 and energy. However, in reverse TCA cycle, two molecules of CO_2 are used to synthesize one molecule of acetyl-CoA using 2 ATP and H^+ from NADH and NADPH (Fig. 12.7a). Two carbon fixing enzymes, pyruvate synthase (pyruvate:ferredoxin oxidoreductase) and 2-oxoglutarate synthase (2-oxoglutarate:ferredoxin oxidoreductase), are the key enzymes of rTCA cycle and were found to be dependent on Fd [61, 62]. Two reactions involving ferredoxin (Fd) significantly help in reversal of some of the reactions, which are otherwise nonreversible in nature. Another enzyme citrate lyase was discovered which is dependent on ATP [63]. ATP-citrate lyase cleaves citrate, a six-carbon compound, into oxaloacetate, a four-carbon compound, and acetyl-CoA. These three enzymes work together to make energetically unfavorable reverse reactions possible. One molecule of succinyl-CoA combines with one molecule of CO_2 to form one molecule of 2-oxoglutarate using 2-oxoglutarate synthase. 2-Oxoglutarate further takes one molecule of CO_2 to form isocitrate using isocitrate dehydrogenase (IDH). Aconitase converts isocitrate to citrate, which is acted upon by citrate lyase to form oxaloacetate and acetyl-CoA. Acetyl-CoA combines with another molecule of CO_2 to form pyruvate using pyruvate synthase. It is followed by synthesis of PEP which combines with another molecule of CO_2 to regenerate oxaloacetate or other intermediates of the cycle in an

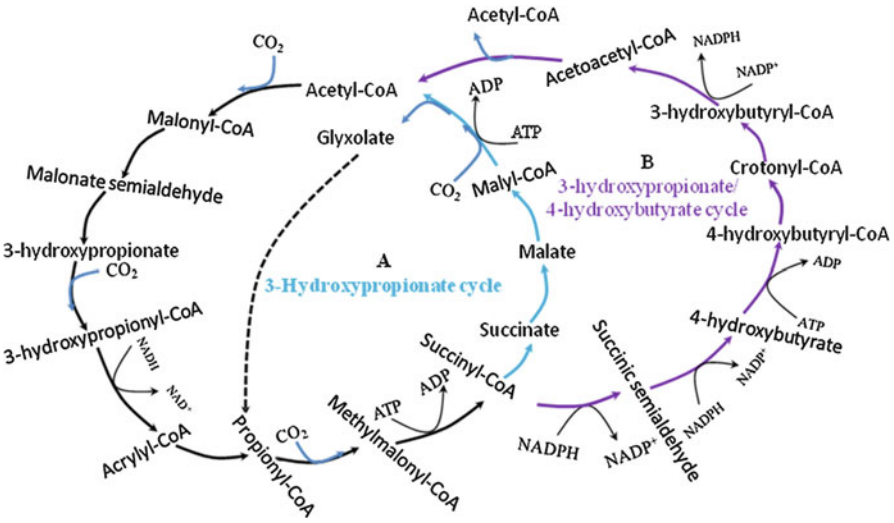

Fig. 12.8 Schematic diagram of (**a**) 3-hydroxypropionate cycle shown in *light blue arrow*, (**b**) 3-hydroxypropionate/4-hydroxybutyrate cycle shown in *purple arrow*. *Black arrows* showing reaction pathways are common to both of them (Reprinted from Ref. [60]. Copyright 2011, with permission from Elsevier)

anaplerotic manner [61]. Shiba et al. (1985) reported direct conversion of pyruvate into oxaloacetate in *H. thermophilus* by an enzyme pyruvate carboxylase [64]. In one complete cycle, four molecules of CO_2 are fixed to generate one molecule of oxaloacetate, which itself is an intermediate of the cycle. It requires two molecules of CO_2, 2 ATP, and 4 NAD(P)H for the formation of one molecule of acetyl-CoA as the end product as shown in Eq. (12.10) [58].

$$2\,CO_2 + 2\,ATP + 4\,NAD(P)H + HS\text{-}CoA \rightarrow Acetyl\text{-}CoA + 2\,ADP + 4\,NAD(P)^+ \quad (12.10)$$

12.3.4 3-Hydroxypropionate Cycle

CO_2 fixation in phototrophic green non-sulfur bacteria takes place using 3-hydroxypropionate pathway unique to eubacterium *Chloroflexus aurantiacus* and some chemotrophic archaebacteria such as *Sulfolobus metallicus, Ignicoccus hospitalis, Acidianus brierleyi*, and *Acidianus infernus* [60, 65, 66]. Enzymes participating in this pathway are not sensitive to oxygen [60, 67]. Acetyl-CoA is the starting precursor of 3-hydroxypropionate cycle. It combines with HCO_3^- to form malonyl-CoA with the help of ATP-dependent acetyl-CoA carboxylase (Fig. 12.8a). Acetyl-CoA carboxylase is also an essential enzyme for the synthesis of fatty acid. It is followed by the conversion of malonyl-CoA to

3-hydroxypropionate by malonyl-CoA reductase in an NADPH-dependent reaction. Malonyl-CoA reductase is a bifunctional enzyme, which reduces malonyl-CoA to 3-hydroxypropionate via malonate-semialdehyde as an intermediate using its aldehyde dehydrogenase and alcohol dehydrogenase domain. Reductive conversion of 3-hydroxypropionate to propionyl-CoA is catalyzed by propionyl-CoA synthase. Propionyl-CoA synthase is a trifunctional enzyme and formally requires three enzymatic reactions. In the first step, activation to 3-hydroxypropionyl-CoA is catalyzed by CoA ligase, which is followed by dehydration of 3-hydroxypropionyl-CoA to acrylyl-CoA by an enoyl-CoA hydratase. Finally, acrylyl-CoA is reduced to propionyl-CoA by an enoyl-CoA reductase using NADPH [65, 68, 69]. Propionyl-CoA undergoes carboxylation, catalyzed by ATP-dependent propionyl-CoA carboxylase to form methylmalonyl-CoA. Isomerization of methylmalonyl-CoA takes place in two sequential steps, catalyzed by methylmalonyl-CoA epimerase and methylmalonyl-CoA mutase to form succinyl-CoA. Succinyl-CoA transfers CoA for malate activation and forms succinate and malyl-CoA [65, 68, 69]. Malyl-CoA, a four-carbon compound, breaks down by malyl-CoA lyase to regenerate starting acetyl-CoA and glyxolate. Carboxylations of acetyl-CoA and propionyl-CoA are the main CO_2 fixation reactions. It is to be noted that actual substrate for both the carboxylation reactions is HCO_3^- rather than CO_2. Each turn of cycle results in net fixation of two molecules of bicarbonate to produce one molecule of glyxolate. Glyoxylate is considered as the initial CO_2 fixation product. Glyxolate is further utilized in the synthesis of the cellular material. Intermediate 3-hydroxypropionate is the characteristic of the cycle, which on reductive conversion produces propionyl-CoA [65, 68, 69]. In 3-hydroxypropionate cycle, six molecules of ATP and four molecules of NAD(P)H are required to reduce two molecules of CO_2 to form one molecule of acetyl-CoA as shown in Eq. (12.11) [58].

$$2\,CO_2 + 6\,ATP + 4\,NAD(P)H + HS\text{-}CoA \rightarrow Acetyl\text{-}CoA + 6\,ADP + 4\,NAD(P)^+ \quad (12.11)$$

12.3.5 Other Pathways

12.3.5.1 Dicarboxylate/4-Hydroxybutyrate Cycle

Dicarboxylate/4-hydroxybutyrate cycle is the newly discovered autotrophic pathway for carbon dioxide fixation in *Ignicoccus hospitalis* (*Desulfurococcales), Thermoproteus neutrophilus*, and an anaerobic member of *Thermoproteales* [70–72]. Evidence of dicarboxylate/4-hydroxybutyrate cycle has been reported by Ramos-vera et al. (2009) in *T. neutrophilus* [72]. This pathway can also be divided into two parts: the first part involves formation of succinyl-CoA from acetyl-CoA using two inorganic carbons and the second part deals with regeneration of acetyl-CoA from succinyl-CoA. Thus, this pathway has similarity with both

reductive tricarboxylic acid cycle (rTCA) and 3-hydroxypropionate/4-hydroxybutyrate cycles (Fig. 12.7b). The first part of the cycle involves the intermediates of reductive tricarboxylic acid cycle (rTCA) and the second part involves the intermediates of 3-hydroxypropionate/4-hydroxybutyrate cycle via the route similar to 4-hydroxybutyrate pathway to succinyl-CoA using pyruvate synthase and pyruvate carboxylase, as carboxylating enzyme. So, the only difference between this and the 3-hydroxypropionate/4-hydroxybutyrate cycle is the way succinyl-CoA is created.

12.3.5.2 3-Hydroxypropionate/4-Hydroxybutyrate Cycle

Another pathway has been reported in some bacteria called 3-hydroxypropionate/4-hydroxybutyrate cycle. This pathway was found in aerobic autotrophic members of Sulfolobales. 3-Hydroxypropionate/4-hydroxybutyrate pathway was discovered in *Metallosphaera sedula* which was earlier believed to fix CO_2 using 3-hydroxypropionate cycle. Malyl-CoA lyase, an enzyme used in the regeneration of acetyl-CoA, was absent in the cell extract of *M. sedula*, resulting in the proposed alternative pathway for the regeneration of starting material of cycle. Therefore, 3-hydroxypropionate and 3-hydroxypropionate/4-hydroxybutyrate cycle share the same steps from acetyl-CoA to succinyl-CoA (Fig. 12.8b). However, the enzymes involved in the reaction steps from acetyl-CoA to succinyl-CoA are not same in both cases. Detailed structure of acetyl-CoA carboxylase participating in 3-hydroxypropionate cycle reveals that the enzyme is composed of four subunits. Contrary to this, acetyl-CoA carboxylase taking part in 3-hydroxypropionate/4-hydroxybutyrate cycle has only three subunits. Similarly, malonyl-CoA reductase of both the cycles is not the same. They differ in the different intermediates through which regeneration of acetyl-CoA takes place from succinyl-CoA. The intermediates between acetyl-CoA and succinyl-CoA in 3-hydroxypropionate/4-hydroxybutyrate cycle are succinic semialdehyde, 4-hydroxybutyrate, 4-hydroxybutyryl-CoA, crotonyl-CoA, 3-hydroxybutyryl-CoA, and acetoacetyl-CoA, formations of which are catalyzed by succinyl-CoA reductase, succinate semialdehyde reductase, 4-hydroxybutyryl-CoA synthetase, 4-hydroxybutyryl-CoA dehydratase, crotonyl-CoA hydratase, 3-hydroxybutyryl-CoA dehydrogenase, and acetoacetyl-CoA β-ketothiolase, respectively [60].

12.4 Enzymes for CO_2 Sequestration

Carbonic anhydrase (CA) is zinc metalloenzyme which catalyzes conversion of free CO_2 into bicarbonates and protons. It is one of the fastest known enzymes catalyzing 10^4–10^6 reactions per second [73]. It has been found in a wide number of living beings such as plants, animals, and microorganisms as they have been found growing well in CO_2-rich conditions, and CA was found essential for this purpose

[74, 75]. In human body, CA is present in the erythrocyte and converts poorly soluble CO_2 in aqueous solution, such as blood plasma to water-soluble bicarbonate (HCO_3^-) anion [76]. Human isozyme HCA II having molecular mass of 30,000 is the fastest CA known so far, having a hydration rate of 1.4×10^6 molecules of CO_2 per second per molecule of CA [19]. Some of the microalgae which have been found containing CA are *Chlamydomonas reinhardtii, Scenedesmus obliquus, Dunaliella tertiolecta, Chlorella saccharophila, Chlorella vulgaris, Chlorella pyrenoidosa,* and *Chlorococcum littorale* [77]. BCA (bovine carbonic anhydrase) has been found stable in wide range of pH (5–10) and temperature (up to 70 °C) [9]. Sulfate and zinc have been found to enhance CA activity.

There are contradictory reports on the effect of CO_2 concentration on the activity of CA in photosynthetic microorganisms such as plants, green algae, and cyanobacteria. In most of the reports, activity of CA isolated from algae is inversely proportional to the CO_2 concentration. It was explained with the fact that RuBisCO can catalyze both oxygenase and carboxylase activities depending upon the nearby CO_2 concentration [29]. CA helps in increasing the concentration of CO_2 near the RuBisCO site. CA can enhance the internal CO_2 concentration up to 1,000 times higher than the external fluid. Therefore, at lower CO_2 concentration, expression of CA increases to maintain carboxylase activity of RuBisCO which in turn reduces atmospheric CO_2 into cellular constituents such as starch, lipid, and protein. However, in few reports, activity of CA initially increases with increase in CO_2 concentration and later starts decreasing with further increase in CO_2 concentration, thus making bell-shaped curve [20]. The reason behind the decreasing trend of CA at higher CO_2 was postulated due to feedback inhibition by bicarbonate and/or decrease in pH at this concentration.

Extracellular and intercellular crude extract of enzyme CA II isolated from *Chlorella vulgaris* were 72 and 160 mg $CaCO_3$ per milligram of protein, comparable to 225 mg $CaCO_3$ per milligram of protein with the purified enzyme from *Citrobacter freundii* [78].

12.4.1 Mechanism of CO_2 Captured by CA

Hydration of CO_2 into solid carbonate such as calcium carbonate is a natural process. There are five reactions required for the transformation of CO_2 into minerals [9, 19, 76].

1. Dissolution of gaseous CO_2 in liquid (Eq. 12.12)
2. Hydration of aqueous CO_2 into carbonic acids (H_2CO_3) (Eq. 12.13)
3. Dissociation of carbonic acid into bicarbonate ions (HCO_3^{2-}) and protons (Eq. 12.16)
4. Dissociation of bicarbonate ions into carbonate ions (CO_3^-) (Eq. 12.17)
5. Reaction of carbonate ions and calcium to form solid calcium carbonate (Eq. 12.18)

12.4.1.1 CO_2 Dissolution

$$CO_{2(g)} \leftrightarrow CO_{2(aq)} \tag{12.12}$$

12.4.1.2 Carbonic Acid Formation

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \tag{12.13}$$

$$K_2 = \frac{k_2}{k_{-2}} = \frac{6.2 \times 10^{-2} s^{-1}}{23.7\, s^{-1}} = 2.6 \times 10^{-3}$$

Where k_2, k_{-2} and K_2 are forward rate, reverse rate and equilibrium constants respectively. As shown in Eq. (12.13), hydration of CO_2 to carbonic acids is the rate limiting step having very less forward reaction constant 6.2 $\times$ 10^{-2} s at 25 °C [18]. However, catalysis by CA increases the rate of reaction manifold showing the high substrate specificity of this enzyme [19]. Enzyme catalyzes the CO_2 hydration reaction in the following two half reactions (Eqs. 12.14 and 12.15) [79]:

$$E - Zn - H_2O \leftrightarrow E - Zn + OH^- + H^+ \tag{12.14}$$

$$E - Zn - OH + CO_2 \leftrightarrow E - Zn - CO_3{}^- + H^+ \rightarrow E - Zn - H_2O + CO_3{}^- \tag{12.15}$$

The above reaction was found dominating when the pH is higher than 10 while it was found negligible at pH less than 8 [80].

12.4.1.3 Bicarbonate Formation

$$H_2CO_3 \leftrightarrow H^+ + HCO_3{}^- \tag{12.16}$$

$$K_3 = \frac{k_3}{k_{-3}} = \frac{8 \times 10^6\, s^{-1}}{4.7 \times 10^{10}\, s^{-1}} = 1.7 \times 10^{-4}$$

Where k_3, k_{-3} and K_3 are forward rate, reverse rate and equilibrium constants respectively. Bicarbonate formation reaction is diffusion controlled and very fast in nature.

12.4.1.4 Carbonate Formation

$$HCO_3{}^- \leftrightarrow H^+ + CO_3{}^{2-} \tag{12.17}$$

$$K_4 = \frac{k_4}{k_{-4}} = \frac{8 \times 10^6\, s^{-1}}{4.7 \times 10^{10}\, s^{-1}} = 4.69 \times 10^{-11}$$

Where k_4, k_{-4} and K_4 are forward rate, reverse rate and equilibrium constants respectively.

12.4.1.5 Calcium Carbonate Reaction

$$Ca^{2+} + CaCO_3{}^{2-} \leftrightarrow CaCO_3 \downarrow \tag{12.18}$$

Calcium carbonate precipitates quickly at the saturation concentration of calcium and carbonate ions. Therefore, continuous supply of carbonate ions by hydration of CO_2 and water is requisite [19]. CA was also found to enhance the calcium carbonate precipitation reaction [9]. However, contrary to enhanced hydration reaction, higher concentration of CA did not increase $CaCO_3$ precipitation reaction [9].

Some of the approaches which have been applied for improving the performance of CA are (1) isolating CAs from thermophilic microorganisms, (2) use of protein engineering to create thermotolerant enzymes, and (3) immobilizing the enzyme for stabilization and confinement to cooler regions and process modification that minimize the stresses such as cooling of the flue gas [81].

12.4.2 Immobilization of CA

Use of CA in aqueous solution has many disadvantages such as reusability and recovery. Immobilization of the CA in the nanoparticles significantly improves the catalytic property, storage, and thermal stability of the enzyme. Immobilized BCA and HCA enzymes were found retaining nearly 90 % of enzymatic activity for more than 20 cycles. Carbonic anhydrase (CA) can be immobilized on functionalized and metal nanoparticles confined mesoporous silica for CO_2 hydration and its sequestration to $CaCO_3$ [76, 82]. Surface-modified magnetic nanoparticle is one of such attractive templates for enzyme immobilization. Enzyme can be easily recovered from a reaction medium by applying a static magnetic field near the immobilized CA in the reactor [76, 82]. Inert materials such as chitosan and sodium alginate are widely used for immobilization of enzymes and microorganism. Whole cells of *Pseudomonas fragi, Micrococcus lylae*, and *Micrococcus luteus* 2 were immobilized on different biopolymer matrices [83]. Bovine carbonic anhydrase (BCA) was covalently immobilized by Vinoba et al. (2012) onto OAPS (octa(aminophenyl)silsesquioxane)-functionalized Fe_3O_4/SiO_2 nanoparticles by using glutaraldehyde as a spacer [76]. Immobilization of CA can be done in many ways such as adsorption on surfaces, entrapment within matrices, and cross-linking within polymeric scaffold [80]. Polyurethane foam is a highly porous hydrophilic polymeric material where enzyme immobilization was easy and fast. CA had 100 % activity over time, and thus reusability was not a concern [80].

12.4.3 Application of CA

Bovine CA has been proposed to inject into wellbore of geological formations to prevent CO_2 leakage through it [84]. CO_2 sequestration by mineral carbonation can use CA to make the process feasible at large scale. Alkaline silicates are abundant and high enough to sequester all the CO_2 emitted from total fossil fuels. Alkaline silicates can dissolve to provide cations in acidic conditions. However, high alkalinity is required for the increase of the rate of gaseous CO_2 dissolving into the carbonate ions CO_3^{2-}. Therefore, enhancing the release of divalent cations from the alkaline silicates and enhancing alkalinity are some of the challenges of the process. Increase in alkalinity increases the rate of dissolution of CO_2 into the carbonate ions CO_3^{2-}, and acidic conditions release the divalent cations required for the formation of carbonates [17]. Denitrification, methane production, and sulfate reduction are some of the alkalinity-producing metabolic processes. Therefore, these processes enhance the carbonation process. Integrating acid-producing process for silicate dissolution and alkaline-producing process for carbonate precipitation together can be used for CO_2 mitigation using biological mineral carbonation. Dupraz et al. (2009) has experimentally shown the use of *Bacillus pasteurii* as a model carbonate precipitating bacteria on the geological sequestration of CO_2 and its transformation into solid carbonate phases [85].

12.4.4 Challenges in Use of CA for CO_2 Sequestration

Enzyme works better at optimum temperature, pH, and enzyme concentration [83]. At lower pH, carbonates prefer to be in dissolved state than the precipitated form, while at higher pH, carbonate ions form but concerns about economical and environmental aspect arise [19]. Lifetime and activity of enzyme greatly depend upon pH, temperature, other ions such as CN^-, and higher concentration of SO_x and NO_x [19]. Higher cost of enzyme and its large-scale production are other bottlenecks to overcome to make the process successful in reality.

12.5 Conclusions

Imbalance between CO_2 emission and sink is the reason for steep rise in the earth's atmospheric CO_2 concentration. This may be the reason for the rise in global mean temperature, causing melting of glaciers, rise in sea level, ocean acidification, unpredicted climate changes, etc. Several biological processes are available for CO_2 mitigation. Carbonic anhydrase (CA) enzyme, algae, cyanobacteria, bacteria, and terrestrial plants are some of the biological methods used for CO_2 sequestration. However, these processes are slow and limited in application. Identifying limiting

parameters and application of technology can accelerate the natural processes manifold. For example, CO_2 hydration, the limiting step in the transformation of gaseous CO_2 to solid bicarbonates, can be accelerated by catalyzing the reaction using CA. Forest and oceanic fertilization can be applied to enhance the photosynthetic efficiency of terrestrial plants and oceanic phytoplanktons, respectively. Similarly, algae and cyanobacteria can be exploited for CO_2 sequestration as they can be grown efficiently at higher CO_2 concentration with higher photosynthetic efficiency.

Acknowledgments The authors gratefully acknowledge the Council of Scientific and Industrial Research (CSIR), Govt. of India, for senior research fellowship and Department of Biotechnology (DBT) and Ministry of New and Renewable Energy (MNRE), Govt. of India, for the financial support. The authors also acknowledge Ms. Pallavi Sinha for reviewing the manuscript.

References

1. Tans P (2010) Trends in atmospheric carbon dioxide. In: Earth System Research Laboratory Global Monitoring Division. http://www.esrl.noaa.gov/gmd/ccgg/trends. Accessed 15 May 2013
2. http://climate.nasa.gov/evidence/
3. Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2:805–814
4. Stewart C, Hessami M (2005) A study of methods of carbon dioxide capture and sequestration–the sustainability of a photosynthetic bioreactor approach. Energy Convers Manage 46:403–420
5. Sharma A, Bhattacharya A, Shrivastava A (2011) Biomimetic CO_2 sequestration using purified carbonic anhydrase from indigenous bacterial strains immobilized on biopolymeric materials. Enzyme Microb Technol 48:416–426
6. Oelkers EH, Cole DR (2008) Carbon dioxide sequestration: a solution to a global problem. Elements 4:305–310
7. Kumar K, Dasgupta CN, Nayak B et al (2011) Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour Technol 102:4945–4953
8. Lee JW, Li R (2001) A novel strategy for CO_2 sequestration and clean air protection. In: Proceedings of fire national conference on carbon sequestration, Washington, DC, 14–17 May 2001
9. Mirjafari P, Asghari K, Mahinpey N (2007) Investigating the application of enzyme carbonic anhydrase for CO_2 sequestration purposes. Ind Eng Chem Res 46:921–926
10. Bachu S (2000) Sequestration of CO_2 in geological media: criteria and approach for site selection in response to climate change. Energy Convers Manage 41:953–970
11. Falkowski P, Scholes RJ, Boyle E et al (2000) The global carbon cycle: a test of our knowledge of earth as a system. Science 290:291–296
12. Hall DO, Woods J, House J (1992) Biological systems for uptake of carbon dioxide. Energy Convers Manage 33(5–8):721–728
13. Whitmarsh J, Govindjee (1999) The photosynthetic process. In: Singhal GS, Renger G, Sopory SK, Irrgang K-D, Govindjee (eds) Concepts in photobiology: photosynthesis and photomorphogenesis. Narosa Publishers/Kluwer, New Delhi/Dordrecht, pp 11–51
14. Benemann JR (1993) Utilization of carbon dioxide from fossil fuel-burning power plants with biological systems. Energy Convers Manage 34(9–11):999–1004
15. Skjånes K, Lindblad P, Muller J (2007) BioCO$_2$ – a multidisciplinary, biological approach using solar energy to capture CO_2 while producing H_2 and high value products. Biomol Eng 24:405–413

16. Loubiere K, Olivo E, Bougaran G et al (2009) A new photobioreactor for continuous microalgal production in hatcheries based on external-loop airlift and swirling flow. Biotechnol Bioeng 102(1):132–147
17. Salek SS, Kleerebezem R, Jonkers HM et al (2013) Mineral CO_2 sequestration by environmental biotechnological processes. Trends Biotechnol 31(3):139–146
18. Druckenmiller ML, Maroto-Valer MM (2005) Carbon sequestration using brines of adjusted pH to form mineral carbonates. Fuel Process Technol 86:1599–1614
19. Bond GM, Stringer J, Brandvold DK et al (2001) Development of integrated system for biomimetic CO_2 sequestration using the enzyme carbonic anhydrase. Energy Fuels 15:309–316
20. Ramanan R, Kannan K, Sivanesan SD et al (2009) Bio-sequestration of carbon dioxide using carbonic anhydrase enzyme purified from *Citrobacter freundii*. World J Microbiol Biotechnol 25:981–987
21. Beedlow PA, Tingey DT, Phillips DL et al (2004) Rising atmospheric CO_2 and carbon sequestration in forests. Front Ecol Environ 2(6):315–322
22. Norby RJ, DeLucia EH, Gielen B et al (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. Proc Natl Acad Sci U S A 102:18052–18056
23. Norby RJ, Wullschleger SD, Gunderson CA (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683–714
24. Griffin KL, Seemann JR (1996) Plants, CO_2 and photosynthesis in the 21st century. Chem Biol 3(4):245–254
25. Karnosky DF (2003) Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. Environ Int 29:161–169
26. Tognetti R, Cherubini P, Innes JL (2000) Comparative stem growth rates of Mediterranean trees under background and naturally enhanced ambient CO_2 concentrations. New Phytol 146:59–74
27. Idso SB, Idso KE, Garcia RL et al (1995) Effects of atmospheric CO_2 enrichment and foliar methanol application on net photosynthesis of sour orange tree (*Citrus aurantium*; Rutaceae) leaves. Am J Bot 82:26–30
28. Manning DAC, Renforth P (2013) Passive sequestration of atmospheric CO_2 through coupled plant-mineral reactions in urban soils. Environ Sci Technol 47:135–141
29. Moroney JV, Somanchi A (1999) How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation. Plant Physiol 119:9–16
30. Tsai DDW, Ramaraj R, Chen PH (2012) Growth condition study of algae functions in ecosystem for CO_2 bio-fixation. J Photochem Photobiol B Biol 107:27–34
31. Yang H, Xu Z, Fan M et al (2008) Progress in carbon dioxide separation and capture: a review. J Environ Sci 20:14–27
32. Oren R, Ellsworth DS, Johnsen KH et al (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–471
33. Yuan H, Ge T, Wu X et al (2012) Long-term field fertilization alters the diversity of autotrophic bacteria based on the ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) large-subunit genes in paddy soil. Appl Microbiol Biotechnol 95:1061–1071
34. Munoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815
35. Kumar K, Sirasale A, Das D (2013) Use of image analysis tool for the development of light distribution pattern inside the photobioreactor for the algal cultivation. Bioresour Technol 143:88–95
36. Hanagata N, Takeuchi T, Fukuju Y et al (1992) Tolerance of microalgae to high CO_2 and high temperature. Phytochemistry 31:3345–3348
37. Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed systems design and performances. Biotechnol Prog 22:1490–1506
38. Lee JS, Lee JP (2003) Review of advances in biological CO_2 mitigation technology. Biotechnol Bioprocess Eng 8:354–359

39. Castenholz RW, Waterbury JB (1989) Group I. Cyanobacteria. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 1710–1727
40. Garofalo R (2009) Algae and aquatic biomass for a sustainable production of 2nd generation biofuels. AquaFUELs Taxon Biol Biotechnol, pp 1–258
41. Garlick S, Oren A, Padan E (1977) Occurrence of facultative anoxygenic photosynthess among unicellular and filamentous cyanobacteria. J Bacteriol 129:623–629
42. Srirangan K, Pyne ME, Chou CP (2011) Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresour Technol 102:8589–8604
43. Giordano M, Beardall J, Raven JA (2005) Mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu Rev Plant Biol 56:99–131
44. Zeng X, Danquah MK, Chen XD et al (2011) Microalgae bioengineering: from CO_2 fixation to biofuel production. Renew Sust Energy Rev 15:3252–3260
45. Cannon GC, Heinhorst S, Kerfeld CA (2010) Carboxysomal carbonic anhydrases: structure and role in microbial CO_2 fixation. Biochim Biophys Acta 1804:382–392
46. Coleman JR (1991) The molecular and biochemical analysis of CO_2 concentrating mechanisms in cyanobacteria and microalgae. Plant Cell Environ 14:861–867
47. Kaplan A, Reinhold L (1999) CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu Rev Plant Physiol Plant Mol Biol 50:539–570
48. Leegood RC (2002) C4 photosynthesis: principles of CO_2 concentration and prospects for its introduction into C3 plants. J Exp Bot 53(369):581–590
49. Badger MR, Price GD (2003) CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J Exp Bot 54(383):609–622
50. Price GD, Maeda S, Omata T et al (2002) Modes of active inorganic carbon uptake in the cyanobacterium *Synechococcus* sp. PCC7942. Funct Plant Biol 29:131–149
51. Spalding MH (2008) Microalgal carbon dioxide concentrating mechanisms: *Chlamydomonas* inorganic carbon transporters. J Exp Biol 59(7):1463–1473
52. Hu JJ, Wang L, Zhang SP et al (2011) Enhanced CO_2 fixation by a non-photosynthetic microbial community under anaerobic conditions: optimization of electron donors. Bioresour Technol 102:3220–3226
53. Nelson DL, Cox MM (2005) Lehninger principles of biochemistry, 4th edn. W.H. Freeman & Co, New Jersey
54. Madigan MT, Martinko JM, Parker J (2003) Brock: biology of microorganisms. Prentice Hall, Upper Saddle River
55. Calvin M, Benson AA (1948) The path of carbon in photosynthesis. Science 107:476–480
56. Kumar K, Das D (2012) Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors. Bioresour Technol 116:307–313
57. Kumar K, Roy S, Das D (2013) Continuous mode of carbon dioxide sequestration by *C. sorokiniana* and subsequent use of its biomass for hydrogen production by *E. cloacae* IIT-BT 08. Bioresour Technol 145:116–122
58. Fast AG, Papoutsakis ET (2012) Stoichiometric and energetic analyses of non-photosynthetic CO_2-fixation pathways to support synthetic biology strategies for production of fuels and chemicals. Curr Opin Chem Eng 1:1–16
59. Müller V (2003) Bacteria energy conservation in acetogenic bacteria. Appl Environ Microbiol 69(11):6345–6353
60. Saini R, Kapoor R, Kumar R et al (2011) CO_2 utilizing microbes – a comprehensive review. Biotechnol Adv 29:949–960
61. Atomi H (2002) Microbial enzymes involved in carbon dioxide fixation. J Biosci Bioeng 94(6):497–505
62. Evans MC, Buchanan BB, Arnon DI (1966) A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc Natl Acad Sci U S A 55:928–934
63. Sintsov NV, Ivanovskii RN, Kondrateva EN (1980) ATP-dependent citrate lyase in the green phototrophic bacterium, *Chlorobium limicola*. Mikrobiologiia 49:514–516

64. Shiba H, Kawasumi T, Igarashi Y et al (1985) The CO_2 assimilation via the reductive tricarboxylic acid cycle in an obligately autotrophic, aerobic hydrogen-oxidizing bacterium, *Hydrogenobacter thermophilus*. Arch Microbiol 141:198–203
65. Alber BE, Fuchs G (2002) Propionyl-coenzyme A synthase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO_2 fixation. J Biol Chem 277:12137–12143
66. Burton NP, Williams TD, Norris PR (1999) Carboxylase genes of *Sulfolobus metallicus*. Arch Microbiol 172:349–353
67. Strauss G, Fuchs G (1993) Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. Eur J Biochem 215:633–643
68. Herter S, Fuchs G, Bacher A et al (2002) A bicyclic autotrophic CO_2 fixation pathway in *Chloroflexus aurantiacus*. J Biol Chem 277:20277–20283
69. Hügler M, Menendez C, Schagger H et al (2002) Malonyl-coenzyme A reductase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO_2 fixation. J Bacteriol 184:2404–2410
70. Berg IA, Ramos-Vera WH, Petri A et al (2010) Study of the distribution of autotrophic CO_2 fixation cycles in Crenarchaeota. Microbiology 156:256–259
71. Huber H, Gallenberger M, Jahn U et al (2008) A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic archaeum *Ignicoccus hospitalis*. Proc Natl Acad Sci U S A 105:7851–7856
72. Ramos-Vera WH, Berg IA, Fuchs G (2009) Autotrophic carbon dioxide assimilation in *Thermoproteales* revisited. J Bacteriol 191:4286–4297
73. Heck RW, Tanhauser SM, Manda R et al (1994) Catalytic properties of mouse carbonic anhydrase V. J Biol Chem 269:24742–24746
74. Karlsson J, Clarke AK, Chen ZY et al (1998) A novel a-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2. EMBO J 17(5):1208–1216
75. Kusian B, Sultemeyer D, Bowien B (2002) Carbonic anhydrase is essential for growth of *Ralstonia eutropha* at ambient CO_2 concentrations. J Bacteriol 184(18):5018–5026
76. Vinoba M, Bhagiyalakshmi M, Jeong SW et al (2012) Carbonic anhydrase immobilized on encapsulated magnetic nanoparticles for CO_2 sequestration. Chem Eur J 18:12028–12034
77. Miyachi S, Iwasaki I, Shiraiwa Y (2003) Historical perspective on microalgal and cyanobacterial acclimation to low and extremely high CO conditions. Photosynth Res 77:139–153
78. Li L, Fu M, Zhao Y et al (2012) Characterization of carbonic anhydrase II from *Chlorella vulgaris* in bio-CO_2 capture. Environ Sci Pollut Res 19:4227–4232
79. Brauer M, Perez-Lustres JL, Weston J (2002) Quantitative reactivity model for the hydration of carbon dioxide by biomimetic zinc complexes. Inorg Chem 41:1454–1463
80. Ozdemir E (2009) Biomimetic CO_2 sequestration: 1. Immobilization of carbonic anhydrase within polyurethane foam. Energy Fuels 23:5725–5730
81. Savile CK, Lalonde JJ (2011) Biotechnology for the acceleration of carbon dioxide capture and sequestration. Curr Opin Biotechnol 22:818–823
82. Vinoba M, Yoon Y, Nam SC et al (2012) CO_2 conversion to $CaCO_3$ by immobilized carbonic anhydrase. KIC News 15(2):32–40
83. Sharma N, Nayak RK, Dadhwal VK et al (2011) Diurnal and seasonal variation of measured atmospheric CO_2 at Dehradun during 2009. In: International archives of the photogrammetry, remote sensing and spatial information sciences, volume XXXVIII-8/W20, 2011 ISPRS Bhopal 2011 workshop, Bhopal, 8 Nov 2011
84. Mahinpeya N, Asghari K, Mirjafari P (2011) Biological sequestration of carbon dioxide in geological formations. Chem Eng Res Des 89:1873–1878
85. Dupraz S, Ménez B, Gouze P et al (2009) Experimental approach of CO_2 biomineralization in deep saline aquifers. Chem Geol 265:54–62