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REVIEW ARTICLE

CO_2 sequestration: microalgae genome analysis and its application of effective green source technology

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Abstract

Microalgae genome technology for CO_2 sequestration is an appropriate vehicle for articulating the importance of the current need and solution for reduction of CO_2 at the atmospheric level. In comparison with C4 plants, microalgae have greater capability to fix atmospheric CO_2. The rate of CO_2 fixation differs in different strains of microalgae. The photosynthetic enzyme RuBisCO is widely responsible for photosynthetic carbon assimilation in all plants including phototrophic algae. The gene *rbcL* encodes this enzyme. The catalytic activity of carbonic anhydrase achieves the CO_2 generation in the RuBisCO. CAH3 gene is essential for generating CO_2 concentration for RuBisCO by dehydration of accumulated inorganic carbon. There are also few other microalgae genes which involves for carbon assimilation. Genomic resource databases and several other nucleotide databases are being used for sequencing the microalgal genomes. Even though, recent advances in genomic studies are providing thrust to enhance the research on microalgal species, they are expensive and resources available for microalgal genomic studies are limited. This review article attempts first as a combined revise on microalgae CO_2 sequestration in the field of basic science, applied aspects, and the role of specific gene(s) in the algal system is well defined which could be a supportive involvement of carbon dioxide reduction as "Green-Gene Technology". This Green biotechnology could be used for Global warming reduction as well as creating wealth from the waste through valuable by-products from the selected microalgae strains in future.

Keywords

carbon assimilation, carbonic anhydrase, genomic resources, microalgal genomes, RuBisCO

Abbreviations

IPCC- Intergovernmental Panel on Climate Change, MOFs- metal organic frameworks, CS-H - Calciumsilicate-Hydrate, Pg C - petagram carbon, AlInGaP II - aluminum indium gallium phosphide, CCM – CO2 concentrating mechanism, GHG-Greenhouse gas, ROS - reactive oxygen species, NGS - Next Generation Sequencing, CRISPR-Cas9 - Clustered Regularly Interspaced Short Palindromic Repeats-Crispr associated protein 9, RuBisCO -EPYC1-RuBisCO-Essential Pyrenoid Component 1, SBPase -Sedoheptulose-1,7-bisphosphatase, FBPase -Fructose 1,6-bisphosphatase, GGT- Green Genome Technology. TALEN-Transcription Activator-Like Effector Nucleases.

Introduction

Climatic change is one of the long term processes which causes changes in weather patterns. It will determine the local, global and regional climate of an earth's surface. Climate change is influenced by the utilization of fossil fuels and greenhouse emissions through deforestation, agriculture and other protuberant causes (1). Climate change occurs naturally due to fluctuation in solar irradiance, discrepancy in orbital parameters of earth and volcanic activities. Generally, some amount of solar energy that enters the earth reflects to the space. Atmospheric gases trap some amount of outgoing solar energy, due to which the temperature gets warmer. If natural heat grabbing/grasping/deceiving properties are unavailable, then the earth's average surface temperature would be less than 33°C. Heat energy is deceived by some amount of gases called greenhouse gases (GHG). In recent decades, especially after the industrial revolution, a sharp increase in atmospheric greenhouse gases is evident mainly due to human intervention. A sharp level of increase in the number of greenhouse gases in the atmosphere results in the increase of earth temperature and alteration of energy which is referred to as global warming (2). The main sources of global warming are anthropogenic activities and the emission of greenhouse gases. A conflict arose when there was a sharp increase of atmospheric greenhouse gases due to several activities of humankind at an alarming rate for the past two centuries. Based on human enhanced global warming effect, according to 2004, about 8 billion tons of carbon dioxide were pumped and the effect of thermal radiation was obstructed due to increased level of GHG. Over the last century, the planet has experienced a high amount of increase in surface temperature. From the years 1906 to 2006, the average temperature of earth's surface was about 0.6 to 0.9 °C, but in the in past 5 years the level of earth temperature was doubled. During the 20th century a rise of about 0.17 m was evident in sea levels. These changes in temperature changes primarily due to the presence of greenhouse gases emitted from various sources. An enormous amount of methane is produced in landfills, animal ordure and agricultural decomposition. Various nitrogen- based fertilizers extricate nitrous oxide into the atmosphere including urea, diammonium phosphate. Once they are discharged, these GHG remain in the atmosphere for decades. According to IPCC 35% increase in CO_2 and 148% increase in methane levels are evident since 1750. Among those GHG, CO_2 gas is at the topmost level; a high amount of CO_2 is released from burning fossil fuels (3). Thus, there is a need to reduce atmospheric carbon dioxide. Researchers, scientist and government throughout the world have been trying to find effective CO_2 sequestration methods by various innovative ways. Currently, this issue is in limelight, which urges researchers to find out effective technologies to capture the major greenhouse gas carbon dioxide. Amid those innovative techniques, microalgae green genome technology plays crucial role in targeting gene for CO_2 sequestration by the activity of microalgae.

The Current scenario in CO_2 sequestration – worldwide

Metallic organic frameworks

Metallic organic frameworks are one of the auspicious methods in CO_2 mitigation i.e. Carbon Capture and Storage (CCS). For this method, materials having high adsorptions and storage capacity are needed to be used. The adsorbent materials utilised to capture CO_2 from flue gas should possess chemical stability, easy productivity with minimal energy utilization- and be economically feasible. Metal Organic Frameworks (MOF's), highly crystalline porous materials constructed by metal ions and organic ligands, proved to be a meritorious adsorbent material for carbon capture. However, eminent advancement in MOF materials for CO_2 capture has been emerged in the past, assessed accordingly, but new inventions are constantly exposed as the field widens quickly (4).

Industrial carbon capture

In industries, carbon capturing techniques are divided into four major techniques, which includes; pre-combustion, post-combustion, oxy-fuel combustion and electrochemical separation. For reducing CO_2 emissions several new power generation concepts have been developed recently. The new concepts also adopt the techniques such as pre-combustion, post-combustion, chemical looping combustion and oxy-fuel combustion (5-7). (I) In pre-combustion capturing technology, a new gasification technique can be used in order to capture the CO_2 prior to burning to generate the power and also to produce combustible gas. The rate of CO_2 concentration is high in the pre-combustion process, which creates a greater driving force and leads to the separation of CO_2. Though it is a well-described enabling technology, it requires equipments like water gas shift and gasification reactors for capturing process though it is a well described enabling technology. (II) The post-combustion is one of the methods which has high potential to retrofit them to the coal power plants (8-13). In the post-combustion process, the CO_2 is captured when the fossil fuel is completely burnt. For capturing NOx (Nitrogen oxides) and SOx (Sulfur oxides), the CO_2 capturing post-combustion technology can be retrofitted to the coal-burning power plants. Absorption, adsorption and membranes are the most important post-combustion CO_2 capture technologies. There are also there other technology necessary for the post-combustion process, it includes absorption, adsorption and membrane filters (III). Due to loss of CO_2 during the process of absorption and solvent regeneration, the post-combustion method requires high energy to complete the process. Oxy-fuel combustion is the process of burning fossil fuel in oxygen-rich gas; it results in the emission of steam and CO_2 which leads to an easier process of capturing CO_2. In this process, a high concentration of CO_2 in flue gas occurs, and due to the absence of nitrogen, the emission of NOx is eliminated. Due to NOx elimination, the combustors are smaller, which collects only a small amount of gas. Therefore pure oxygen combustion is complicated than air combustion. The process requires large cryogenic air separation units because it avoids high-temperature combustion and 60% of flue gas is recycled back to the combustor. Advanced oxygen

separation membranes can be used to lower the energy requirements (14).

CO_2 uptake mechanism in cement-based materials

In cement-based materials the carbonation reaction occurs chemically between CO_2 and cement hydrates, which is an essential reaction for CO_2 uptake by a cement-based material. The process of carbonation is considered as a weathering degradation of the cementitious composite, because of the possibility of the decomposition of CS-H (calcium silicate-hydrate) phases in the cementitious composites (15). Owing to these factors, the process of carbonation was considered as a negative influence affecting the durability of concrete (16). However, studies carried out over the years revealed that, the process of carbonation does not always have a negative influence as it may also contribute to enhancing the durability as well as the mechanical properties of the material. As a result, several researchers have suggested that the source material could be potentially used for CO_2 uptake (16-19). Even though an adequate level of understanding has been obtained in the carbonation mechanism of cement-based materials, the distinctiveness and differences in the intrinsic carbonation mechanism should be studied in detail and summarized in comparison to other CO_2 uptake methods (17).

Types of carbon sequestration

The process of carbon sequestration involves capturing and long term storage of industrial effluents containing carbon, which in-turn aids in reducing the emission of carbon into the atmosphere in the form of CO_2 (1) in Fig. 1.

Abiotic sequestration

The term abiotic sequestration refers to the process in which sequestration is carried out without the intervention of living organisms such as plants and microbes. This type of sequestration is performed by using only engineering techniques, physical and chemical reactions. In comparison with biotic sequestration, abiotic sequestration has a greater sink capacity which has received considerable attention in geological and oceanic structures (20) (21). Prompt studies are being carried out in developmental and experimental technologies for capturing, transporting and injecting CO_2 (22).

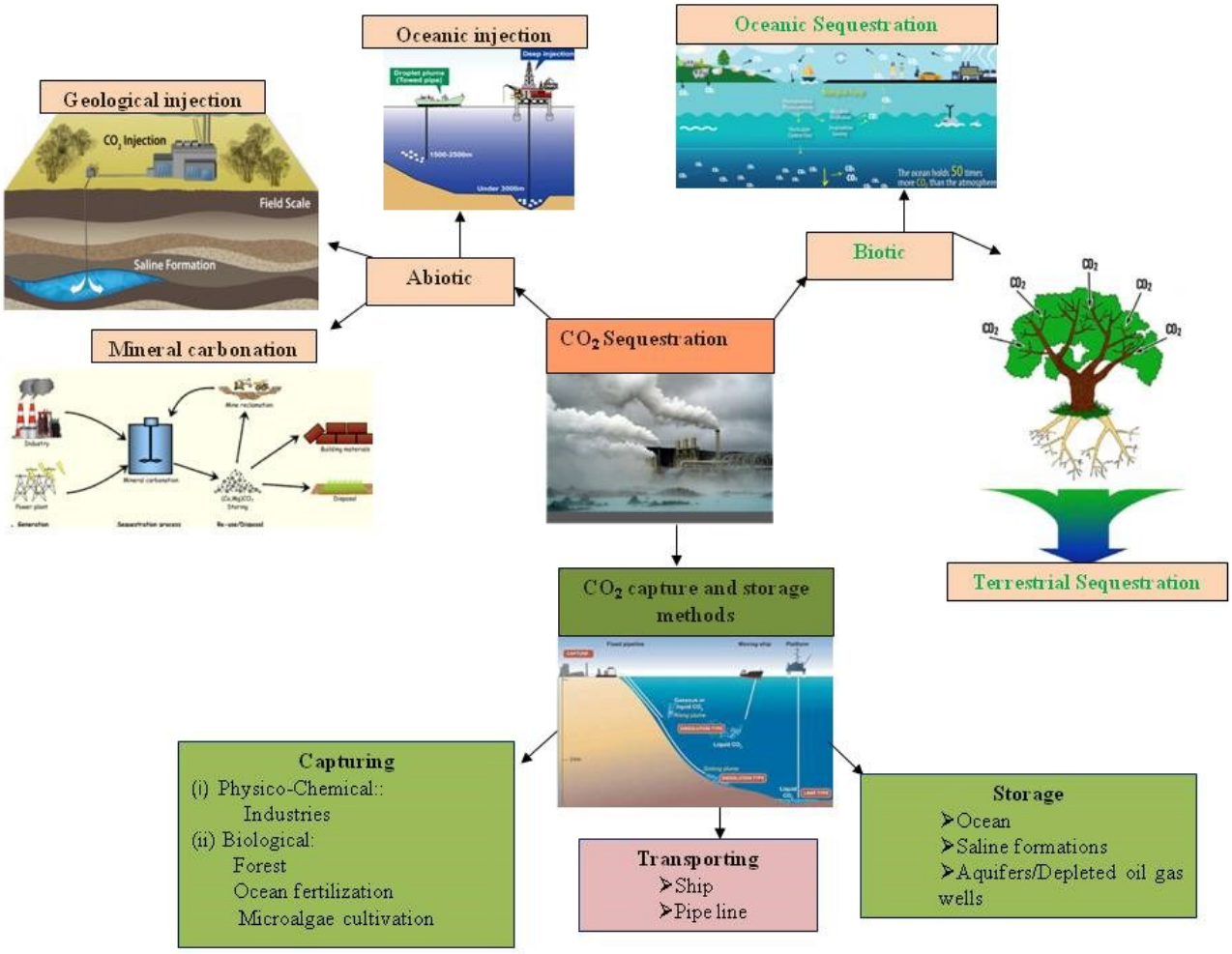

Fig. 1. General types of CO_2 sequestration and its capture methods.

Oceanic injection

In the late 1970s, the initial proposal of injecting CO_2 into the oceans was started; after that, substantial progress of injecting CO_2 into the ocean was made. This technique requires the injection of CO_2 at great depths into the ocean to stabilize and minimize the gassing and it has been widely practised by engineers for about three decades. Techniques used to inject liquefied CO_2 obtained from industrial sources are as follows; liquefied CO_2: (i) should be lighter than water and has tends to rise approximately to 1000 m depth, forming a droplet plume and hence is injected below 1000 m from a manifold lying on the ocean floor, (ii) it

should be injected at a depth of 500-1000 m with a denser mixture of CO_2 and seawater which helps it to sink deeper into the ocean, (iii) discharged into the ocean using large pipes dragged behind ships, and (iv) pumped onto the bottom of the ocean floor into depression causing the formation of a CO_2 lake. The injection of liquefied CO_2 at approximately 3000 m depth into the ocean helps to achieve stable oceanic sequestration (23). The estimated value of carbon sequestration by oceanic sink capacity is 5000-10000 Pg C (peta-gram carbon), which helps to decrease the net CO_2 emission released into the atmosphere (21).

Geological injection

This technique involves the injection of industrial CO_2 into the geological layers, which includes places such as saline aquifers, old oil wells- to increase yield, stable rock strata and coal seams (24-26). This entire process starts from the capture, liquefaction, transportation and injection of CO_2 into the deep geological layers. There are many concerns raised regarding the geological sequestration which includes; (i) leakage of CO_2, (ii) safety concerns regarding the storage of vast quantities of CO_2, and (iii) the cost of the whole process. Saline aquifers are located in the underground strata below the freshwater reservoirs. They are made up of very porous sediments filled with brackish (saline) water (27, 28). Despite the concerns, people have argued that the risks of leakage is low by the presence of an impermeable layer in between the saline aquifers and the freshwater reservoirs. Hydrodynamic sequestration takes place after the CO_2 is pumped into the saline aquifer which reacts with dissolved salts and forms carbonate. The CO_2 which is injected into the sea displaces the liquid brine because of its lower viscosity and density. Therefore the CO_2 is injected in supercritical state. A multiphase-multicomponent environment is created when the CO_2 is injected in situ as it dissolves in the aqueous phase and forms a gas-like phase. An economical strategy to enhance oil recovery (EOR) is injecting CO_2 into the oil or gas reservoirs where the CO_2 displaces the gas and oil. This process of CO_2 enhanced recovery has been found to be advantageous to increasing the oil production and gas fields (29). Despite the drawbacks and concerns, this technique of CO_2 sequestration is being carried out in the United States, within the state of Texas, to inject 20 million Mega gram (Mg) of CO_2 yr^{-1} at a price of \$10 to \$15 Mg^{-1} per year (30).

Scrubbing and mineral carbonation

Scrubbing is a common method used for capturing carbon. This method involves the chemical absorption of carbon dioxde using a carbonate solvent or an amine (21). Further, the captured CO_2 is purified by passing it through an absorption column containing solvents like amine, nickel and other elements such as ceramic-based compounds, lithium silicate, and K_2CO_3. By passing the captured CO_2 through a absorption column having solvents like nickel, amine and elements such as ceramic-based compounds like lithium silicate and K_2CO_3, the CO_2 is filtered further (31). The CO_2 gas is transformed into mineral carbonates such as $MgCO_3$, $CaCO_3$ and other minerals, which are thermodynamically and geologically stable. However, as the mineral carbonates formed are stable rocks, the CO_2 sequestered forever within them need to be concentrated more on for developing a clear effective technology.

Biotic sequestration

Arbitration of higher plants and microorganisms in removing CO_2 from the atmosphere is known as biotic sequestration. This process varies from managing process, but it helps in the reduction of offset emissions. Biotic resources like water and energy are used in managing the terrestrial C pool process. Few other types of biotic sequestration are explained below.

Oceanic sequestration

Carbon sequestration in the ocean is achieved using biological processes, mainly photosynthesis. Phytoplankton is one such mechanism (32), which fixes approximately 45 Pg C yr^{-1} (Peta gram carbon stock per year) (33). From the phytoplankton, several organic materials get deposited on the ocean floor and get sequestrated (34). Among those organic factors, 'Fe' acts as a limiting factor, therefore various studies are insisted to find out the significance of the 'Fe' factor on biotic sequestration of CO_2 in the ocean (33, 35, 37).

Terrestrial sequestration

Terrestrial carbon sequestration involves the transfer of atmospheric CO_2 into biotic and pedologic Carbon pools. In the atmosphere 8.5 Pg C yr^{-1} is emitted but anthropogenically emitted 3.5 Pg C is only retained in the atmosphere which owe to terrestrial carbon sinks and the sequestered CO_2 plays a crucial role in the global C cycle. In terrestrial sequestration, the sequestration process takes place in terrestrial ecosystem where the C sink occurrs through photosynthesis and the CO_2 is stored in living and dead organic matter. Terrestrial carbon sequestration can also be called as win-win or no-regrets strategy (38) due to various benefits like improving the quality of soil and water, increasing the yield of the crop and restoring the degraded ecosystem. This type of sequestration provides numerous benefits without affecting the global climate (21).

CO_2 capture and storage methods

Physicochemical carbon capture and sequestrations strategies are collectively assorted as carbon capture and storage (CCS) methodologies. It operates by three major steps which includes CO_2 capture, CO_2 transportation, CO_2 storage. CO_2 is captured from huge sources like power plants and cement manufacturing plants. Consecutive methods are adopted for the capture and separation of CO_2 from other exhaust components such as chemical absorption, physical adsorption, membrane separation, cryogenic distillation (39-41). The highly concentrated CO_2 collected is then constricted and transited through pipelines or shipped to repositories (42, 43). Finally the acquired CO_2 is gathered into reservoirs, such as geological storage, oceanic storage wherein the CO_2 is directly inoculated deep into the ocean, saline formations, aquifers and depleted oil or gas wells (30). The economically high operation, conveyance and environmental threat of long term CO_2 leakage and other uncertainties (44, 45) are some of the major drawbacks.

Physicochemical CCS methods are victorious in trapping CO_2 from point sources generating high concentra-

tions of carbon di oxide. But the sources from diffused, non-point emissions and low concentrations of CO_2 cannot be captured (46, 47). Besides physical and chemical CCS, there is a biological route that capture CO_2 through natural sinks. Biological capture methods includes; i) forestation; afforestation, reforestation, and the farming of crops and livestock (38, 48). (ii) ocean fertilization; fertilizing oceans with iron and other nutrients promoting increased carbon dioxide uptake by the phytoplanktons (49) and (iii) microalgae cultivation (44, 48, 50-52).

The Critical role of microalgae in regulating atmospheric carbon dioxide sequestration

The oldest and significant group of organisms on earth is microalgae. Microalgae is photoautotrophic primitive plant that shows rapid growth and ranges few microns in size. Many algal species have oil substances in it and their weight ranges between 20-50% of dry biomass. 1kg dry algal biomass make use of 1.3 kg of CO_2 (53, 54). Approximately half of the atmospheric oxygen on the earth is produced by microalgae, while consuming vast amounts of the greenhouse gas CO_2. Since algae produces huge amount of energy and biomass, it is used as a source of food, feed, fuel, stabilizing agent and waste water treatment. Algae generate a large amount of biomass and energy. Due to its rapid growth ability it has the efficiency to fix CO_2 which is ten folds better than terrestrial plants. The potential of photoautotrophic algal cultures play a major role in diminishing the release of atmospheric CO_2 which help in alleviating the trend towards global warming. The selection of optimal microalgae is crucial to recognize workable CO_2 biological fixations systems. Specific strategies required for CO_2 sequestration influence the selection of optimal microalgae. The production of biomass of microalgae have higher potential to reduce the carbon dioxide emissions and increase the level of world energy supply. Microalgae has greater potential to tolerate high temperature and CO_2, therefore nowadays carbon sequestration is done using microalgae with maximum efficiency. Microalgae can assimilate CO_2 within various ranges of concentration by selecting competent species. The use of microalgae for CO_2 sequestration also yields several byproducts and shows versatile performance (55, 56).

CO_2 tolerating microalgal species based on parameter

Light and Light sources

Biological processes such as microalgal growth, photosynthesis, carbon dioxide fixation etc., are highly dependent on the intensity of light (57, 58). In Calvin-Benson cycle, the enzyme RuBisCo (1,5 Bisphosphate Carboxylase Oxygenase) fixes the CO_2 by using the ATP (Adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate) which are produced in the light reactions during photosynthesis. High light intensity produces high reactive oxygen species which can cause abnormal physiological reactions due to oxidative stress, while in low light intensity CO_2 fixation and biomass concentration are low (59). These high reactive oxygen species cause the decline of CO_2 during photosynthesis because it affects the essential proteins which are needed for electron transfer. The ability of microalgae to photo acclimate to different intensities of light helps them to carry out the usual metabolic reactions and physiological processes (60). Different species have different photo acclimation periods (61, 62). One of the main factors for the cultivation of microalgae is light. It can be both natural and artificial. Light sources such as light-emitting diodes, AllGaP ll (Alluminium Indicum Gallium Phosphide) having 613 mm of wavelength, halogen, fluorescent, incandescent etc. can also be used, out of which usage of AllnGaP ll and Light-emitting diodes are efficient and Cost-effective (63). Recent studies have found new sources of illumination and controlling the intensity of light which includes; (i) illumination with selected wavelengths of light, (ii) use of dye compounds which helps to lower the energy of photons, and (iii) light filters such as nanoparticles and fluorescent paints (64). Research on the cultivation of microalgae have been carried out using artificial light under controlled conditions as well as large scale outdoor cultivation using solar light, both depending on the location, climate, season and circadian cycle (65). To The exposure to light can be both continuous or with interrupted photoperiods. A successful cultivation system is a system that produces high biomass along with high cell concentrations which causes different gradients of light in the medium and the cells are exposed to different light intensities which in turn helps to minimize the cost of production (66, 67). To achieve a decent amount of CO_2 fixation, the cells should be exposed to a certain period of dark-light cycles with a ratio of 18:6 or 12:12 cyclic patterns (64). The algal growth and its ability to fix CO_2 is affected by either the light intensity or through the light-dark cycle (58).

Temperature

In the cells, the metabolic processes and the availability of physicochemical CO_2 is influenced by temperature (68). Carbon dioxide solubility is indirectly proportional to temperature; it is identified that when the temperature is (>20 ^{0}C) lower is the CO_2 solubility. In spite of that, when temperature increases the RuBisCO's affinity for carbon dioxide decreases (69). Nevertheless, the effect of temperature on the metabolic reaction rate is strain-dependent (70) experimentally reviewed that the effect of temperature using different species, by indicating adaptations of different species to high temperature and biochemical effects. Generally, under the temperature between 15-30 °C with optimal values at 20-25°C the microalgae grows.

Mechanisms of carbon fixation and metabolic activity are affected below 16 ^{0}C. On the other hand, the higher the temperature lower is the carbon dioxide solubility (71) and many species will die when the temperature is above 35 ^{0}C (72), and it also decreases the growth rate by increasing both respiration and photorespiration. Light and temperature are considered as the greatest important factors which affects the biomass productivity and CO_2 fixation.

pH

For the dispensation of different dissolved inorganic species (CO_2, HCO_3^-,CO_3^{2-}), the pH is regulated on the chemical level. It was reported that pH is an extensive factor that determines algal growth by affecting the various enzyme

activity (73, 74). Moreover, the value of pH has a powerful impact on the growth of cells and the level of optimum or tolerating level are species-dependent. The optimum pH is in the range between 6-8.3 which is neutral to slightly alkaline (75-79). Growth takes place, there will be equilibrium condition is dissolved inorganic carbon species, CO_2 dissolution and CO_2, nitrate uptake cause fluctuation in pH. However, CO_2 input concentration influence these changes. Usage of HCO_3 causes the zin metalloenzymes (carbonic anhydrase) to convert into CO_2 which results in liberation of OH (hydroxyl ions) and increase in pH. It shows that limitations in CO_2 effects the performance of the system, but supplying CO_2 will control the optimum level of pH in the culture. Reports are on maintaining an optimum level of pH by supplying CO_2 rich gases to the culture (80). This method was used widely in order to meet the carbon demands for micro algae cultures which shows a high rate of CO_2 fixation and huge production of biomass.

Dissolved O_2 concentration

For measuring photosynthesis activity in microalgae, dissolved O_2 are used; the resulting value is greater than the values obtained under the equilibrium condition of air. Dissolved O_2 are mostly consumed by heterotrophic microorganisms and low dissolved O_2 point out complication in the growth of micro algae. Dissolved oxygen may exceed 250 % of saturation during daytime (58, 81). Excessive concentration of oxygen causes oxidative stress in ROS and content against with CO_2 help rubisco for fixing CO_2. The concentrations of O_2 and CO_2 determines the process of photosynthesis (carboxylation) and photorespiration (oxygenation). Low ratio level of O_2 /CO_2 diminish the photosynthetic rate in micro algae. Thus, CO_2 fixation favours photorespiration CO_2 release (58).

CO_2 concentration

CO_2 fixation includes solubilization (from gas to liquid phase) and mass transfer, ionic equilibrium condition (CO_2, HCO_3-, CO_3^{2}-) and carbon ingestion by the microalgal cells. The strain has a direct influence on tolerance and optimal CO_2 concentration (82) and most microalgae have the potential to grow well at 2% CO_2, but levels above 5% CO_2 (83) may cease their cell growth (84, 85). This effect may result in acidification of the stroma in the chloroplast (82) and abolition in important enzymes involved in Calvin-Benson cycle. It was reported that rate of CO_2 fixation is high in different micro algae like *Nannochloropsis oculata, Botryococcus braunii*, *Scenedesmus obliques, Chorella vulgaris* and *Synechococcus* sp. by the effect of different level of CO_2 (86). However, it was found that the maximum biomass production obtained in painting the CO_2 tolerant species like *Scenedesmus* sp. (80%), *Euglena gracilis* (45%) and *Chlorella* sp. T-1(100%) when the concentration of CO_2 ranges between 5%-20% (87). Maximum growth at 10% CO_2 was seen in *Chlorella* sp. KR-1 and tolerated up to 70% CO_2 (88), *Spirulina* sp. (MCRC-A0003) maximum growth and 30–

Table 1. CO_2 tolerating microalgal major contributing families containing species under optimum condition

Major CO_2 tolerating Family	Microalgae	Temperature (°C)	Irradiance (µmol m–2 s–1)	pH	CO_2 tolerance (%)	Conical flask / Photobioreactor	Growth rate (day–1)	Biomass productivity (g L–1 day–1)	CO_2 biofixation (mg L–1 day–1)	Mixing	References
Chlorophyceae	*Chlorella* sp	27	100	7	40	Conical flask, 8 days continuous aeration, air + CO_2; horizontal bubble column, 11 days continuous aeration	0.38	0.09	7.2	Aeration	(90, 91)
	Scenedesmus almeriensis	35	200	7-8	10	Tubular photobioreactor outdoor condition flue gas (pure CO_2) on demand operated continuously	0.34	0.42	790		(58)
	Scenedesmus obtusiusculus	35	300	7-8	10	Bubble column, 14 days, continuous aeration, air + CO_2	0.34	0.52	970[a]		(58, 93)
	Dunaliella sp.	25	100	8	5	Horizontal bubble column, 11 days continuous aeration aeration N_2+ CO_2	0.25	0.12	10.4		(91)
	Dunaliella salina (DCCBC2)	27	80	8	10		0.42	0.13	8.2		(91)
	Haematococcus pliviallis	25-28	90	7	34	Bubble column, aeration, air + CO_2	1.29				(94-96)
Bacillariophyceae	*Phaeodactylum triscornutum*	20.4	10	7-8	15	Erylenmeyer flask, aeration Roux bottles 11 days, continous aeration air + CO_2		0.15	280[a]		(97)
Cyanophyceae	*Spirullina platensis*	30	330	9-10	10	Bubble column, 25 days, continuous aeration	0.65	0.15	280[a]		(98-101)
	Spirullina maxima	35		9-10		Container of glass, 15 days, mechanical agitation	0.6	0.15	280[a]		(60, 99)
Euglenaceae	*Euglena gracilis*	27-31	100	7.8	45	Photobioreactor, 28 days flue gas 11% CO_2 continous operation	0.31	0.29	74	Fermentor	(102, 103)

Source adopted and modified (65).

[a]Calculated from the biomass productivity, according to the equation: CO_2 fixation rate (P_{CO2}) = 1.88 × biomass productivity (g L^{-1} day^{-1}), which is derived from the molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$

50 % CO_2 reduction (89). The above-mentioned range is significant because some micro algae have the ability to grow well in 10 to 15% of CO_2 this type of CO_2 range is generally found in flue gas even though flue gas is one of the GHG causes pollution but for micro algae it is really good carbon source (Table 1).

CO_2 tolerance of microalgae

In a CO_2 sequestration system the flue gas is directly utilized from the power plants (104) and the cost of separating CO_2 becomes minimum when flue gas is used. High CO_2 tolerant species is adequate when the power plant flue gas contain high concentration of CO_2. There are a few microalgae such as *Cyanidium caldarium* (105) and some other species of *Cyanidium* has the capability to grow in pure CO_2 (106). However the micro algae can grow under concentrations 5% to 45 % of CO_2 better growth was observed in 5% concentration and the micro algae cannot grow under concentration of CO_2 higher than 45% (102). *Scenedesmus* sp. has the capability to grow in 80 % of CO_2 concentrations its maximum cell growth was observed under 10 % to 20% concentrations of CO_2. CO_2 acts as a carbon source for the group development of micro algae. Control the pH (107) of the culture is contributed by CO_2 supply (108). Chemical analysis of algal biomass shows that for 1 kg production of biomass about 1.5 to 2.0 kg of CO_2 is required (109). The important point that should be taken into consideration is that CO_2 should not be allowed to reach higher and lower concentrations (107). The lowest limitation and highest inhibition concentration vary in different species ranging between 2.3×10^{-2} M to 2.3×10^{-4} M (110). According to previous studies, one of the primary difficulties and limitations that must be worked out is the supply of carbon to microalgal mass culture systems (111-113).

CO_2 tolerance mechanism

The mechanistic presumption of the effect of the increased CO_2 concentration on the growth and efficiency of algae was analysed or studied or examined earlier (90, 114, 115). Analysis reveals that *Dunaliella tertiolecta* cells enclose more starch granules with a well-developed pyrenoid under ordinary air than cells grown under high CO_2 cells. The chloroplast was detected neighbouring to PM and the envelope was denser electronically under ordinary air while that in enriched air, the chloroplast were found in the inner area of the cells and the envelope was electronically lighter. The effect of CO_2 analysed in the chloroplast cells was contradictory when it was observed or studied under plasma membrane (116). This implies that microalgae have the potential to endure high concentration of CO_2 by altering their anatomical structure and reorganisation of certain cellular organelles (104, 114).

Role of CO_2 in algae physiology, biochemical pathway and its fixation

CO_2 fixation in microalgae takes place in two phases (i.e.) the light reaction which includes photoactive complexes called photosystem I (PS-I) and photosystem II (PS-II) and the dark reaction also called as the Calvin cycle. The PS-I and PS-II are responsible for transferring the light energy into the electron transport chain through the excited chlorophyll dimer (117), (118). In the light reaction the cells are illuminated by the utilization of light energy to form energy-storage molecules ATP and NADPH, which are used to capture and reduce CO_2 (117).

In the PSII complex, the photosynthetic process takes place when the core P680 chlorophyll dimer reaches adequate excitation energy from the sunlight. And the excited electron is transported to the primary electron acceptor molecule. This course of the process is called photo-induced charge separation. The excited electrons are transported through an electron transport chain to the P700 chlorophyll dimer present in the PS-I. The excited electrons from the P700 chlorophyll dimer are further oxidized into ferredoxin and NADPH by the light excited antenna (118-120).

Energy harvested by means of the light reaction can be used for the phosphorylation of ADP to form ATP, which is called photophosphorylation (121). During the electron transport chain each pair of the electron produces 1.3 ATP molecules (121). The Calvin cycle also called as the dark reaction or carbon-fixation cycle is the second stage of photosynthesis which can function both in the presence and absence of light (117, 121). RuBisCO enzyme present in the Calvin cycle carries out both carboxylase activity and oxygenase activity (low affinity for CO_2). The carboxylase activity of the enzyme along with the utilization of ATP molecules, converts CO_2 into sugar (Fig. 2). Glycolate -2-phosphate is formed as an end product through the activity of the enzyme oxygenase. This glycolate-2-phosphate is responsible for the liberation of CO_2 fixed in the carboxylase activity of RuBisCO and its synthesis consumes significant amounts of cellular energy and this end product is not utilized by the cell.

The microalgae biomass production may reduce approximately 50% due to the oxygenase activity of RuBisCo (69). Many studies have been done with light intensity and quality in terms of light supply which are the variable that impact photosynthetic activity and increase the growth kinetics of microalgae. It was reported that a strain *Scenedesmus obliquus* was studied with range of light/dark frequencies in an exponential condition where the photosynthetic rate is increased and also shows that in the long dark phase, the microalgal cells exploit more light energy than the light phase (122). Nevertheless, the long dark phase need not to be a necessity for attaining a greater rate of photosynthesis, nor do the cells have the ability to acclimatize in both light and dark cycles. The exposure of microalgal cells to a high spectrum of light at longer duration may ultimately damages the protein D1 in PSII. Since the damage of protein D1 causes poor capturing of photons which may lead to a total reduction of photosynthetic activity.

There was a study on the influence of photoperiod by using Blue Green Algae cultures in BGN medium and refinery effluent for CO_2 sequestration; during this experiment, it was found that there is a gradual decrease in the biomass production in BGN medium at longer dark period (123). Refinery effluent used for growing microalgae achieved a photosynthetic quotient of 0.74, meaning that

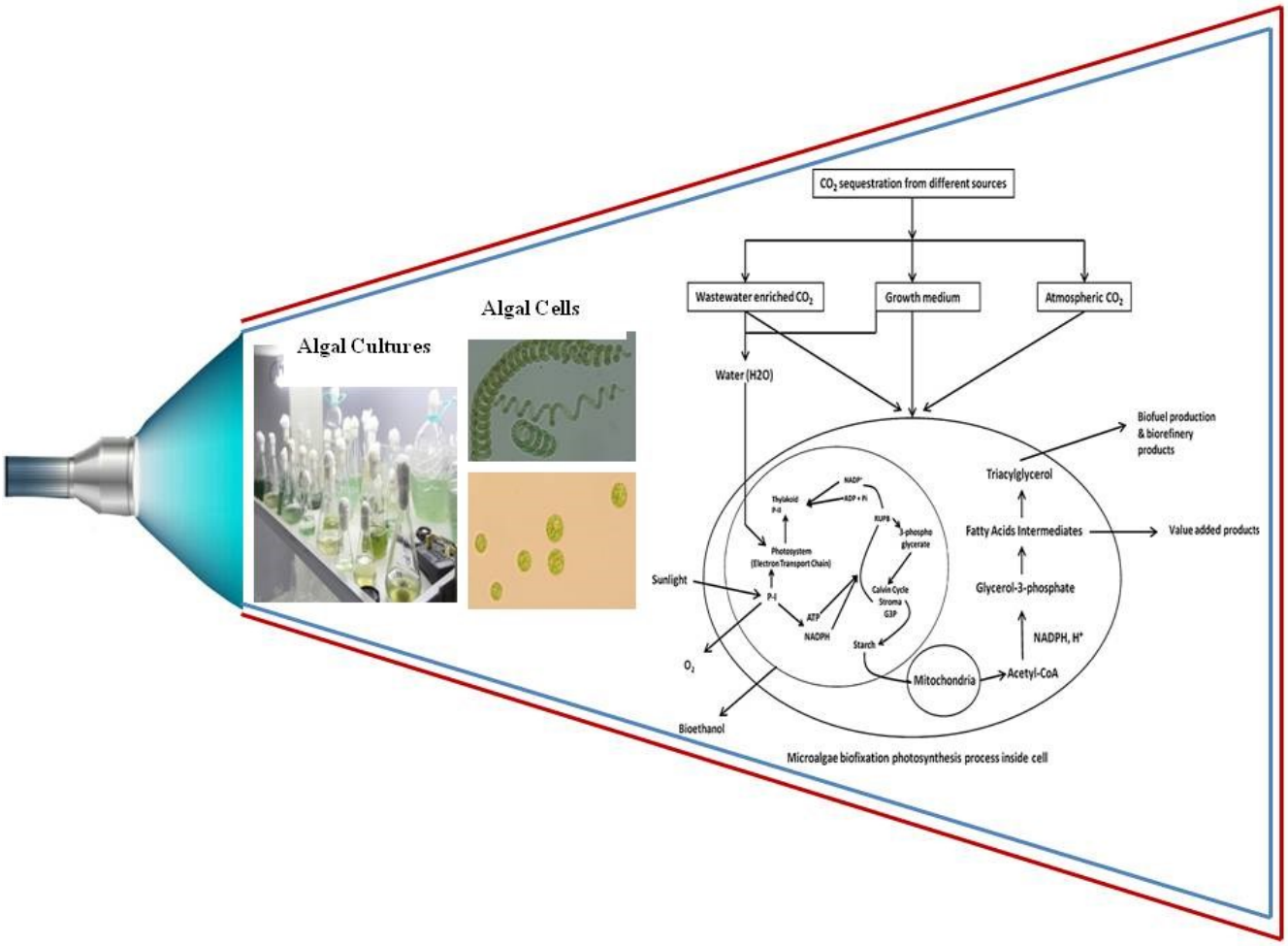

Fig. 2. Microalgal physiological pathway involved in biochemistry of CO_2 fixation Source adapted and modified (55).

1g of CO_2 is utilized to liberate 0.74 g of O_2. The above study confirm that the intermittent life cycle has an impact on the gas exchange pattern of the microalgal system. Sequential changes in the intensity of light and density of culture promotes a high growth rate of microalgae. Low culture density with high exposure of light intensity causes photo inhibition of cells where as in high culture density, the spectrum of penetrating light act as a limiting factor. However, the growth and CO_2 mitigation of microalgal cell can be achieved more when they are exposed to red and blue spectrum of light (124, 125). Microalgae possess a unique mechanism called as CO_2-concentrating mechanism (CCM), in which the cytoplasm of the microalgae innately accumulate inorganic carbon in large quantities, compared to that on the outside. There is an impulsive decrease in the pH of the medium when there is a high concentration of CO_2 due to which the growth of many microalgal species cease. The variation in the pH is due to the increase in the CO_2 mass transfer mechanism from the gas mixture to the medium. Fuel gases are said to contain very high concentrations of CO_2 and at times more than 30% of CO_2. Also, the O_2 produced during photosynthesis inhibits the microalgal growth. Therefore it is very essential to avoid accumulation of CO_2 by routinely removing it to ensure the continuous growth of microalgae (41).

Studies have reported that the cycles and pathways such as Calvin cycle, PEP (Phosphoenolpyruvate) carboxylase or synthetic pathways can improve the rate of CO_2 fixation (126, 127). Researchers have worked on the enzyme RuBisCO via engineering to increase the catalysis rates of carboxylation reaction which in turn enhances the activation state of the enzyme and reduces the oxygenation reaction. Another method to inhibit the oxygenation reaction is to enhance the regeneration phase of the Calvin cycle and CO_2 enrichment around the enzyme. The results from the above investigations revealed that the media lacking CO_2, temperature or light cannot support the proper functioning of RuBisCO enzyme for fixing the carbon flux via Calvin cycle. *Chlamydomonas reinhardtii* is a single-cell green alga that has a heterotrophic life cycle and is capable of attaining the nuclear genome of an MRL1-deficient strain and MRL1 maturation factor at different levels. Due to these factors, *Chlamydomonas reinhardtii* is considered as an ideal candidate for RuBisCO engineering. When compared to the wild type, the deficient strain showed that RuBisCo could maintain phototrophic growth even when it was lowered up to 15 %. These discoveries have proposed that for modifying the accumulation of RuBisCO, inducible MRL1 promoter can be applied based on the light intensity and CO_2 concentration of the culture (126-129) (Table 2).

Life cycle assessment of CO_2 sequestration microalgae

Life cycle analysis (LCA) is one of the systematic ecological

Table 2. Comparison of candidate CO_2 sensitive genes at high vs. low CO_2 concentration using RNA sequence.

Metabolic pathway or biological process Description (Step by Step process)		Candidate CO_2 Sensitive genes	
		Complemented network (present work)	(128)
Transport, chloroplast	↓	DAT1, NAR1.2	
Porphyrin and chlorophyll metabolism	↓		GSA
Carbon fixation	↓	MDH5	RBCS1
Glyoxylate metabolism	↓		GLYK
Glycolysis, gluconeogenesis, valine, leucine and isoleucine degradation	↓	PGK1	PGK1
Glycine, serine and threonine metabolism	↓		GCSP, THS1
Pentose phosphate pathway	↓	TAL1, RPE1, RPI1	RPE1
Mitochondrial transport	↓	MIT28, PTB12, PTB4, PTB2	
Phenylalanine, tyrosine and tryptophan biosynthesis	↓	AST4	
Oxidative phosphorylation	↓	NDA3, IPY1, IPY3	
Extracellular transport	→		PTA3, PTA4

Source adopted and modified (129).

List of candidate CO_2 sensitive genes identified in the metabolic pathway of carbon fixation, occurs in various continuous transport process from chloroplast to extracellular components of microalgae are differentially expressed in a transcriptome dataset previously published comparing cells at high vs low CO_2 concentrations using RNA sequence.

methods used to assess the input and output records of production systems in the entire life cycle of microalgae. This overall process includes the addition of raw materials, product production, usage and disposal of waste materials (130). With the help of this assessment the problem shifting crisis arises during different stages of the life cycle can be detected i.e. utilization of lower energy utilization and high manufacturing cost and also it projects the innovative opportunities and environmental performance based on the specific functional unit of the product (130, 131). In microalgal system the energy balance is calculated by evaluating the energy inputs needed for each LCA stage against the total expressed energy inputs related to specific product or idea of interest. Since balancing energy might influence in any phase of life cycle, it is necessary to monitor every life cycle phase to prevent any misfortunes or implications in rest of the life cycle chain.

In theory, the mechanism of capturing GHG would involve capturing CO_2 flue gas from various power stations which is subsequently utilized for promoting growth and biofuel production in microalgae (132). It was known that capturing the fuel gas does not credits the production of permissible carbon; the fact behind this is the fuel originates from algae is burnt and went back to the atmosphere, it was arise by the replacement of fossil fuels. Supplementary carbon credits may be available if the spent microalgal biomass is used for the production of electricity. Therefore, the biomass of algae would replace the coal, gas or other materials used for production of energy (133). Comprehensive life cycle calculation of energy process should be done for making biofuel. This calculation will help quantify the emission of the GHG at each stage of the process which enables the researchers to find out whether the fuel emits less CO_2 or not compared to fossil fuels (131, 132). For comparing the systems on quantitative basis different microalgae having the same functions should be selected as a unit and all the flows of energy within the system is standardized (134). To evaluate the balancing of CO_2 in a system, it is important to find and collect the details about the total release of CO_2 from fossil energy out of the CO_2 uptake from microalgae cultivation (132). LCA basically covers biomass cultivation, harvesting, lipid extraction and various product formation. However, the treatment of wastewater is not covered in detail. Although, a detailed cost-effective analysis was done on various algal biomass production systems (132, 135). Moreover, combining wastewater treatment and algal biomass production could be a cost-effective technology as well as clean to green energy process at a large scale level, thus reducing pollution.

Application of CO_2 enriched algal biomass

The existing and impending uses of microalgae are diverse and abundant. Various microalgae applications include healthcare, food, feed and industry. Even though the utilization of cyanobacterial species in the field of the food industry was done before hundred years, but new advances had been made in the 20th century (136). Several microalgae species like *Spirulina platensis*, *chlorella sp.* and diatom *Odontella aurita* plays a major role in the market for microalgae as food and food supplements. Additionally, *Dunaliella salina* is used to produce of beta carotene, *Haematococcus pluvialis* used for the production of astaxanthin and *Aphanizomenon flos-aquae* is used as a dietary supplement. The most common species of microalgae such as *Spirulina*, *Chlorella* and *Scenedesmus* could become a vital source of land animal feed. In chicken farming, the effect on the colour of the meat and egg yolk is due to the incorporating of 5 to 10% microalgae in the diet (137). Due to the presence of high-value compounds (HVC) in microalgae helps to produce products related to human health care. Most of the plants and animals doesn't have certain enzymes to produce long-chain polyunsaturated fatty acids (PUFAs), but various marine microalgae produces long-chain PUFA's including Arachidonic (AA), gamma-liolenic (GLA), docosahexaenoic acid (DHA) and Eicosapentaenoic (EPA). Adequate consumption of such fatty acids could have valuable effects on human health. 35–45% DHA is present in the oil from the *Stramenopile, Schizochytrium* sp. (permitted as a food ingredient). In comparison, 10% alpha-linolenic acid,

the precursor of omega-3 is found in most conventional oils rich in omega-3 (walnut oil, canola oil). The production of these PUFAs will undoubtedly be a main dispute in the coming years. Algal pigments, such as carotenoids, beta-carotene, alpha carotene, lutein, lycopene and zeaxanthin are already commercially exploited but are also the subject of intensive research. Moreover to colour salmon 95% synthetic astaxanthin used but in Japan and Canada natural source of astaxanthin extracted from *Hematococcus pluvialis* was used (138). Phycobiliprotiens produced by various microalgae plays a vital role in clinical and immunological therapy purposes (139). Certain microalgae species produce free radicals which results in the formation of Reactive Oxygen Species (ROS). These ROS play an imperative role in numerous chronic diseases or acute relations. Even though, production of these ROS is also employed in human health therapy. However, numerous microalgae applications have been found out in various fields, but still, genomic applications are under construction.

Genomic studies in microalgae for CO_2 sequestration

The acquirement of important genomic data on microalgae since the 1990s is due to the ascend of next-generation sequencing (NGS) technologies. An increase in NGS technologies paved the way for the availability of microbial genomes, in addition to 14 nuclear genomes, the gene repertoire of many additional species is now accessible through transcriptomics. Among the photosynthetic organisms, *Chlamydomonas reinhardtii* (Chlorophyta) was chosen as a model for sequencing its entire nuclear genome in 2007 (140). On the species, genetic and post-genomics tools like RNA, microarrays, antibodies and used over the past few decades. These approaches led to the identification of biological processes in response to stress, metabolic pathway and circadian clock system of microalgae species (141), photosynthetic electron transport chains (142), mechanisms of carbon concentration (143) and flagellar assembly. Also, significant research contributions have been provided by proteomic studies in photosynthesis, molecular biology and evolution (144, 145). Several other sequenced micro algae have been selected based on their phylogenetic distribution, ecological role or nature of harmfulness. These algal species are sequenced to provide extensive information on the evolution of species, aid in identifying the metabolic pathways and other processes involved in various phase of its life cycle. Data containing these genomic sequence information plays crucial role in investigating post -genomic studies, which including proteomic and transcriptomic analyses. In some cases sequencing the full genome of an algal species is excluded due to its larger genome size. Gene catalogues for such species are built by transcriptome sequencing. Transcriptomic data are utilized for exploring phylogenomic and functional post- genomic studies. Constructing these transcriptomic and genomic data took several months or years. These technological development have paved way for chief fundamental research in global ecology, functional biology and evolution of organisms. These revolution in genomic data will speed up well commercialization of algal-based bioactive compounds through the understanding of the fundamental and regulatory pathways of algal cells (146).

Targeting microalgal genes and their function accounting CO_2 capturing

RuBisCO enzyme plays a vital role in CO_2 assimilation of algae (147). The gene rbcL is responsible for the production of this enzyme. This enzyme is found in specific location; carboxysomes in cyanobacteria and in other algae it will be located in pyrenoids. However there are few studies that shows the hypothesis of localization of RuBisCO for attainment of environmental CO_2 (148). RuBisCO is abundant in carboxysomes (149) than pyrenoids. But in microalgae RuBisCO is the major protein component of pyrenoids (148). At the site of RuBisCO enzyme the machinery of cells elevates the CO_2 concentration by increasing the cellular inorganic carbon (Ci) which facilitate the carboxylase activity of RubisCO. Several Ci transporters found in the plasma membranes and chloroplasts are considered as main proteins on the carbon uptake process (12). By anchoring around the pyrenoid tubules the RuBisCO-EPYC1 (Essential Pyrenoid Component 1) enhance the CO_2 fixation (150). Enzyme Fructose 1, 6- bisphosphate aldolase (FBA) involves in the Calvin cycle which leads to CO_2 sequestration. Very recently the over expression of cyanobacterial FBA by engineering the calvin cycle was found to enhance the photosynthetic capacity of *C. vulgaris* (151). On the other hand, the over expression of *Chlamydomonas* SBPase (Sedoheptulose-1,7-bisphosphatase) was found to improve the photosynthetic activity in *Dunaliella bardawil* (152). In microalgae and Photosynthetic microbes the reaction catalyzed by the FBPase (Fructose 1, 6-bisphosphatase) can be targeted to improve the photosynthetic efficiency and accumulation of biomass. CIA5 factor, transporter of Ci and carbonic anhydrases (CA) are considered as the targets of manipulation inorder to increase the photosynthetic performance and eventually biomass yield (153-156). CA is another enzyme that is also involved in CO_2 fixation of algae. It is a zinc metalloprotein found in the periplasmic space of the cell which catalyzes the interconversion of CO_2 and HCO_3- based on the following formula:

$$CO_2+H_2O\leftrightarrow H_2CO_3\leftrightarrow H^++HCO_3^-$$

Carbonic anhydrases will enable the way to accumulate HCO_3- within the cell. Various microalgae growing under limiting CO_2 conditions produced high amount of CA (147). In *C. reinhardtii* and *Dunaliella salina* the enzyme periplasmic CA's encoding genes are identified (157). CA1 is one of the periplasmic CA which are recognized as prominent low CO_2 inducible proteins. These proteins are identified in *C. reinhardtii*. With the help of periplasmic CA microalgae cells use external HCO_3- to do the prices of photosynthesis. For photosynthesis, the usage of external Ci is decreased due to the presence of external CA (148, 158, 159). The periplasmic CA probably increases the efficiency with which the cells can take in external Ci. This includes both the supply of CO_2 for diffusion across the plasma membrane and the supply of HCO_3^- for the plasma membrane's HCO_3^- transport system.

The CAH genes play an important role in cellular carbon uptake (152), (148) and are the targeting genes for CO_2

sequestration. In microalgae the environmental level of CO2 influences the expression of CAHs genes. The main role of these CAHs is catalyzation and inter conversion of CO_2 and carbonic acid ($H2CO_3$) which increases the level of carbon uptake at the site of chloroplast for photosynthesis. A family of enzymes known as the carbonic anhydrases (CAs) have a class of ∝-CAs in them which are responsible for the diffusion of CO_2 across the plasma membrane of the cell. Proteins found to be responsible for the variations in the concentration of CO_2 are CAH1, CAH3, CAH4, CAH5 and CAH6. It has been reported that at low CO_2 levels, a protein known as low-CO_2 inducible protein-A (LCIA) is induced and highly expressed which encodes a nitrate transporter that increases the transport of bicarbonate (HCO_3^-) in the stroma (CIA) (152).

Sensitive gene

Genes that are responsible for the changes involved in carbon fluxes in the system are known as sensitive genes (129, 160). Some examples of the sensitive nodes responsible for the simulations in the metabolic network are H-protein (GCSH), low-CO_2 inducible protein-A (LCIA), phosphoglucomutase (GPM2), E1 component, genes coding for glycerate kinase (GLYK), dual-function alcohol dehydrogenase/ acetaldehyde dehydrogenase (ADH1), carbonic anhydrase-5 (CAH5), NAD-dependent malate dehydrogenase (MDH3), glycine cleavage system and alpha subunit (PDC3).

Selected genes are cultivated in varying concentrations of CO_2 to study their carbon uptake properties. Experimental studies have revealed that, the carbon concentrating mechanism (CCM) related genes when cultivated in low CO_2 condition are over expressed and the cells change their metabolism which enhances the production of enzymes to uptake carbon.

Earlier comparative transcriptomics studies had revealed that when *Chlamydomonas* cells were subjected to a wide range of CO_2 concentrations namely very low (0.02%), low (0.05%) and high concentration (5%) (152) the results showed that the mutant strain cc2702 and wild type strain cc125 vs. acia5 when exposed from low vs. high concentration of CO_2 showed the presence of at least 345 genes that were differentially expressed. When the wild type cells were exposed from very low vs. high concentration of CO_2, they showed the presence of 696 differentially expressed genes (13, 161).

Level of CO_2 optimization in microalgae by specific gene

Microalgal cells exposed to high levels of CO_2 showed increased capability toward biomass production in previous experimental results and also the expressions of the gene transcripts for CAHs such as CAH1, CAH4, CAH5 and LCI1 were increased in low CO_2 concentrations. Microalgae have various advanced modes of CCMs to alleviate RuBisCO and arrest atmospheric CO_2 in a broad range. But one common expression in these mechanisms is the crucial act of the numerous uniquely confined intra and extracellular carbonic anhydrases (162). The specific gene CAH3 codes for the protein/metalloenzyme ‘Carbonic Anhydrase’ whose function is the reversible hydration of CO_2. CAH3 has two major physiological functions. One is that, it is a perfect entity for the CA that formulates the acquired bicarbonate into CO_2 for further fixation. And the other being is the ability to maintain the accumulated manganese of PSII (163, 164).

Genetically modified microalgal strains for CO_2 sequestration

In general, aquatic microorganisms often limits the availability of Ci and CO_2 using CCM's that allow them to optimize carbon acquisition. With the increasing knowledge in mutational approaches and genetic engineering, consolidated image of functional components and molecular details regarding CCM regulation is developing (165). Genetic and metabolomic engineering aids in the beneficial modification of microalgal strains. CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-Crispr associated protein 9), TALEN (Transcription Activator- like Effector Nucleases) and CRISPR- Cas9 (Clustered Interspaces Short Palindromic Repeats - Cripe associated protein 9) are some genome editing tools used currently for gene alterations. Synthetic biology brings in the concept of ‘Biobricks’ to generate artificial regulatory pathways which can alter the metabolism and inturn administer beneficial cellular characteristics. Ribosome binding sites, promotors, terminators, etc are some of the interchangeable units that serve as Biobricks.

The Calvin cycle of *Chlorella vulgaris* was genetically engineered (151) which enhanced its photosynthetic capacity by -1.2-fold (166). This strain mutated by NTG (N-methyl-N’-nitro-nitrosoguanidine) mutagenesis thrived efficiently in 10% CO_2 and is approached for CO_2 sequestration. Some cyanobacteria members were genetically modified to produce and secrete CAs into the medium which was efficiently transformed the CO_2 into HCO_3^- and which in turn was taken up by the microalgae and further fixed into biomass through the process of photosynthesis.

Genomic Resources of microalgae and CRISPR/Cas9 tools

Until 2008, only three microalgal species were sequenced which includes *Chlamydomonas reinhardtii*, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (167). But over the past years, the great revolution of next-generation sequencing technology has created a prompt rise in the availability of complete genomes of various algal species and drafts. Currently about 60 algal accessions are sequenced completely and it's completed genome profiles are available in phytozone and "The Greenhouse”.

There are about three databases available for algal genomics. The first database is pico-PLAZA (https://bioinformatics.psb.ugent.be/pico-plaza/), which consists of genomic informations and instinctual tools of functional genomics of the 16 algal species (168). The second database is Algae Path (http://algaepath.itps.ncku.edu.tw); it gives information about the expression of gene predicting metabolic pathway of *Chlamydomonas reinhardtii* and *Neodesmus* sp. UTE X 2219-4 (169). The third one is ALCOdb (http://alcodb.jp), which provides details about co-expression gene data of two algal species (*Cyanidioachyzon merolae* and *Chlamydomonas reinhardtii*) (170). Additionally, the completed genome sequence draft are also available in phytozome (https://phytozome.jgi.doe.gov) and JGI Ge-

nome portal (https://genome.jgi.doe.gov) (Supplementary Table 1).

Challenges in genomic studies

Bringing out new technologies and products into the market for commercialization faces many restrictions. Genetic engineering is one of the most used technology to augment CO_2 assimilation in microalgae. As genetically engineered organisms such as transgenic or recombinant algae are required to undergo regulatory compliances in the form of laws and policies in many parts of the world, this technology faces several challenges. Due to the challenges faced, even though extended research is carried out to genetically modify and produce an improved and enhanced performing microalgae, their commercialization is restricted. Therefore, advances in the technology can only occur when the investigation/research and policy complement each other.

- Research centres must be well equipped and possess expert human resources who are well trained with hands-on experience in genomic analysis/sequencing and complete the tasks efficiently.
- Defining the full genome sequences and Complementary DNA Sequences (cDNA) of an organism is the foremost step of the scientific community to get whole sequence information about particular organisms, which can be established further by training the agencies and other communities through workshops or tutorials.
- Lack of prior knowledge in the field of microalgal genomics, proteomics and its biochemical-physiological process increases problems during genomic studies (183).
- The path toward algal genomics has yet not touched its horizons, required for pan-genomic studies.
- Phylogenetic and BLAST analysis are the significant tools used effectively for genomic studies, but they only explain the presence, absence and variability among known genetic loci. Multiple species are extensively sequenced to get an understanding of the basics of algal species (184).
- In RuBisCO, the rate of catalytic activity has been studied to improve of photosynthetic CO_2 utilization in the field of genetic engineering. Even though, it resulted in limited success (185, 186).
- Evaluating and correlating useful genomic inputs is very necessary to scrutinize for predicting the function of proteins. There should be awareness among the researchers about the quality of published genome assemblies because most of the genome assemblies are incomplete and inaccurate (187).
- There has been a rapid increase in the completed genomes and the available number of draft of algal species due to the revolution in the past decade on the "next-generation sequencing" technologies.
- Several attempts made to define the genomic sequence of microalgal species of disparate group (160, 167).

Progress in microalgal genome analysis

Substantial advances in the evolution of new genetic manipulation tools helps to manipulate the central carbon metabolism in the microalgae. Most of such advances could be used in defining the industrially relevant organisms (188). Auspicious developmental advances in the metabolic engineering tools not only increases the endogenous carbon storage compounds production like starch and TAGs, it also helps in the direct production of hydrocarbons which can be used in fuel production. The usage of these metabolic engineering tools in microalgae will enhance the production of sources of renewable fuel (188).

C. reinhardtii and *P. tricornutum* are considered as reference organisms because it is used to characterize the specific adaptations of algae at the molecular and genetic level (187). Microalgal species such as *Fragilariopsis cylindrus*, *Pseudo-nitzschia*, *Thalassiosira rotula*, *Botryococcus braunii*, *Chlorella vulgaris*, *Dunaliella salina*, *Micromonas pusilla*, *Galdieria sulphuraria*, *Porphyra purpurea*, *Volvox carteri* and *Aureococcus anophageferrens* are some of the genome sequencing projects in progress (189). Only three microalgal species *Phaeodactylum tricornutum*, *Chlamydomonas reinhardtii* and *Thalassiosira pseudonana* were sequenced till 2008 (167). Due to the technological revolution in genomics, copious number of completed genomes of various algal species were drafted and stored. Review are on the efforts of sequencing the genome of miscellaneous group of microalgal species (160, 167). Cas9 is one of the developing and systematic nuclease guided genome editing tool which helps in enhancement of algal genomics. Nowadays CRISPR/Cas9 is extensively cast-off by researchers because it is easily adaptable and cost-effective (182). The CRISPR/Cas9-based genome-editing method was done in oleaginous microalga *Nannochloropsis oceanica*, using the enzyme nitrate reductase (NR; g7988) to increase the scalable amount of oil production and carbon sequestration (190).

Future prospects

Expanding knowledge on the diversity of algae that can be used as a competent and economical alternative for wastewater treatment, CO_2 fixation, lipid synthesis towards economic biofuel production etc., is essential (191). This can be achieved by combining genomic studies along with technologies such as CRISPR-Cas systems and genome engineering, which will result in exploration and knowledge gaining of a broader range of organisms (187). Pivotal need should be taken as an expectant alternative to current mitigation strategies is an integrated CO_2 bio-fixation, biofuel production and as a value addition for algal biomass.

Conclusion

This review covers the general method of CO_2 sequestration and found that the CO_2 sequestration using Green Genome Technology (GGT) is a very acute method than others be-

cause specific target gene(s) present in microalgae shows the reduction of CO_2 which could enhance the efficacy of the method in a short period. Moreover after CO_2 sequestration, microalgal biomass production can be used to extract various high-value added products and socio-econometric. This will help to improve the economic feasibility of algal by -products from CO_2 enriched biomass and protect climate change. Consciousness about applications of algal biomass could make a common man into an entrepreneur. Currently, microalgae Genome studies have become very popular for CO_2 sequestration, and attempts are under way to Lab science to Land science technology because microalgae genome resources is a quantum jump to development in the efficacy of CO_2 sequestration. This research review article could bring their scope and hope to make the CO_2 sequestration in a combact green genome technology which could be more effective on time and every time emphazising the 4Es that is Education, Environment, Economy and Empowerment of our world legacies.

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Authors contributions

All authors have read and approved the final manuscript. As a corresponding author, I certify that the submission is an original work and is not under review at any other publication, nor has it previously been submitted to any other journal.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interests to declare.

Ethical issues: None.

Supplementary data

Table 1. CRISPR/Cas9 tools.

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