



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## REVIEW ARTICLE

# Maximum-CO<sub>2</sub> Tolerance in Microalgae: Possible Mechanisms and Higher lipid Accumulation

Vaibhav Nagaich\*, Sumeet Kumar Dongre, Pushpendra Singh, Mahavir Yadav, Archana Tiwari  
School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh, Airport Bypass  
Road, Bhopal - 462033 India

### Manuscript Info

#### Manuscript History:

Received: 16 March 2014  
Final Accepted: 23 April 2014  
Published Online: May 2014

#### Key words:

CO<sub>2</sub> fixation, photosynthesis, lipid

#### \*Corresponding Author

Vaibhav Nagaich

### Abstract

Microalgae are belonging to unicellular or simple multicellular photosynthetic microorganisms that have the capability to fix CO<sub>2</sub> from various sources, industrial exhaust gases, with the environment and soluble carbonate salts. Unbalanced production of the environment CO<sub>2</sub> constitutes a most important challenge to worldwide sustainability. Photoautotrophic algal cultures have the possible to lessen the release of CO<sub>2</sub> into the environment by CO<sub>2</sub> fixation, helping alleviate the trend toward global warming. Increased CO<sub>2</sub> concentration improved significantly the growth rate of the species. In this study, the effects of nitrate feeding on microalgal growth and related CO<sub>2</sub> fixation were evaluated, as a affinity to increase carbon fixation. The present study aimed at investigating the mechanisms of carbon dioxide fixation in microalgal cultivation. Biomass composition accessible a prevalence of proteins but also a high amount of lipids. The Rubisco give the high greatest specific reaction rate by the use of high CO<sub>2</sub> and (initially) O<sub>2</sub>-free situation. Photosynthetic equipment state transitions that increase ATP generation, up regulation of H<sup>+</sup>-ATPase pumping protons out of the cell, shutdown of CO<sub>2</sub>-concentrating mechanisms, and alteration of membranes' fatty acid composition are presently assumed to be the key mechanisms leading cellular pH homeostasis and hence microalgae's tolerance to high CO<sub>2</sub> levels. The different percentage of carbon dioxide fixation enhances the higher lipid in the microalgae.

Copy Right, IJAR, 2014., All rights reserved.

## Introduction

Due to the consumption of fossil fuels, the atmospheric greenhouse gases such as carbon dioxide (CO<sub>2</sub>), sulfur dioxide (SO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) rises. (Hempel, Petrick et al. 2012). The unbalance overload of CO<sub>2</sub> in the environment having the major problem for the environment. CO<sub>2</sub> fixation by microalgal cultures has the possible to reduce the release of CO<sub>2</sub> into the environment, helping improve the movement toward global warming (Ono and Cuello 2003).

An raise in atmospheric CO<sub>2</sub>, derived from fossil fuel combustion, poses big challenges to worldwide sustainability (Change 2007). Natural photosynthesis in green plants achieves carbon dioxide (CO<sub>2</sub>) fixation on a worldwide scale. The incorporation of carbon dioxide (CO<sub>2</sub>) into the biosphere by the photosynthetic action of plants and microorganisms has been approximate to total to about 1011 tons of CO<sub>2</sub> per year (Ramanan, Vinayagamoorthy et al. 2012).

With the fast development of present industry, they require for energy has enlarged substantially in current years, and consequently, unconventional energy sources are being explored. At this time Biodiesel is produced from plant and animal oils. Biodiesel is a important fuel that has grown extremely in popularity over the past decade. With

decreasing reserves of fossil fuels, At this time it is more important than ever to search for transportation fuels that can serve as alternatives to crude oil-based fuels such as diesel and gasoline (Sudhakar and Premalatha 2012).

Micro algae can convert CO<sub>2</sub> to potential biofuels, foods, feeds and high-value bioactive compounds by using of sun light (Chisti 2007).

The unbalance overload of carbon dioxide in the atmosphere cause major problem in the environment, the microalgae having the useful phenomena above this problem. Micro algae utilize carbon dioxide as sources of nutrients.

Based on these evidence the current work is an attempt to create biodiesel from microalgae and also to apply algae for carbon dioxide sequestration.

### **Microalgal strains for fixation of CO<sub>2</sub>**

The various microalgal species have been shown to be able to use carbonates such as Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> for cell growth(Huertas, Colman et al. 2000).

Microalgae can fix carbon dioxide from different sources, which can be characterized as

- CO<sub>2</sub> from the environment,
- CO<sub>2</sub> from industrial exhaust gases , and

Fixed CO<sub>2</sub> in the form of soluble carbonates (e.g., NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>) (Wang, Li et al. 2008).

A number of microalgae species have been studied for CO<sub>2</sub> reduction, such as *Chlorella kessleri*, *Scenedesmus obliquus*(de Moraes and Costa 2007) , *Chlorococcum littorale*(Ota, Kato et al. 2009), *Dunaliella tertiolecta*, *Chlorella vulgaris*, *Spirulina platensis*, *Botryococcus braunii*(Sydney, Sturm et al. 2010), *Nannochloropsis oculata*(Chiu, Kao et al. 2009), and *Chlorella sp.*(Chiu, Kao et al. 2008).

### **Photosynthesis and growth**

Photosynthesis is the important mechanism in which living organisms obtains their energy and nutrients, directly and indirectly. Algal photosynthesis is a unique process by which solar energy is transformed into chemical energy that is stored in organic carbon substance through the cycling of atmospheric CO<sub>2</sub>. Photosynthesis takes place in specific organelles called chloroplasts of eukaryotic species, and also in a membrane-bound sac known as a thylakoid of the cyanobacterium due to lack of defined chloroplast structure in the prokaryotic organism. Photosynthesis is commonly conducted in two divide steps—the light and dark reactions(Rubio, Camacho et al. 2003). CO<sub>2</sub> convert in to the organic carbon in algae by two carboxylation pathways C<sub>3</sub> and C<sub>4</sub> carbon fixation pathway. Enzyme Rubisco (ribulose-bisphosphate carboxylase) catalyzes the reaction of RuBP + CO<sub>2</sub> + HO<sub>2</sub> to 2 PGA (phosphoglyceric acid), In the C3 pathway. In Calvin cycle, PGA convert in sugar. It is supposed that mainly algae and higher plants utilize the C3 pathway to fix the inorganic carbon. Some algae and plants evolved another C4 pathway—CO<sub>2</sub> is first transformed into a four-carbon organic compound and then CO<sub>2</sub> released for fixation by Rubisco(Raven, Giordano et al. 2012).

Cell growth is another basic feature of algal cell biology, which, together with photosynthesis and nutrition supplements, determines the maximal potential of the algal biomass production. Cell growth probably consists of two phases—earlier cell proliferation and later improvement in cell volume, both of which directly contribute to algal biomass accumulation(Beardall and Raven 2013).

The photosynthesis is the most useful phenomenon in microalgae, solar energy converted in to chemical energy and the CO<sub>2</sub>released for fixation by Rubisco.

### **Robustness of pH homeostasis in the cell**

The fast inhibition of photosynthesis observed under high-CO<sub>2</sub> circumstances could be a consequence of inactivation of the key enzymes of the Calvin cycle due to acidification of the stromal section of the chloroplast(Zarco-Tejada, Catalina et al. 2013) under high-CO<sub>2</sub> stress. This proposal was confirmed by Pronina et al.(Pesheva, Kodama et al. 1994) who recognized, using vital <sup>31</sup>P-NMR, that the pH of the cytoplasm in the CO<sub>2</sub>-tolerant microalga *C. littorale* does not fall below 7.0, even during farming under excessive (40 %) CO<sub>2</sub> levels(Miyachi, Iwasaki et al. 2003).

### Active transport of H<sup>+</sup>

Taking into account these result, it is obvious that efficient control of intracellular pH is crucial for high-CO<sub>2</sub> tolerance. Such control might be achieved by pumping the protons from the cytoplasm into the vacuoles, for example, via utilization of the surplus ATP generated as a result of cyclic electron transport by the ATPase and H<sup>+</sup>-pyrophosphates associated with the tonoplast(Rienmüller, Dreyer et al. 2012).

A significant factor of CO<sub>2</sub> tolerance is the ability of microalgae cells to enhance the pH of the medium through active growth via nitrate uptake. Alkalization of the medium is assumed to recompense, to a substantial extent, for the acidification effect of high CO<sub>2</sub> concentrations. This mechanism operates in *Emiliana huxleyi*(Fukuda, Suzuki et al. 2011).

A sufficient supply of NO<sub>3</sub> - would be essential for proficient acclimation to high CO<sub>2</sub> and carbon capture from flue gases by the microalgal cultures.

### Shutdown of the CO<sub>2</sub>-concentrating mechanism (CCM)

(Sato, Kurano et al. 2002) established a drop in intracellular pH within 1 h of transferring *C. littorale* cells growing in low CO<sub>2</sub> to 40 % CO<sub>2</sub>. This effect was eliminated by action of the cells with ethoxzolamide. Farming of the low-CO<sub>2</sub>-adapted microalgal cells at eminent CO<sub>2</sub> bring about a substantial refuse in CCM activity. In *Chlamydomonas reinhardtii*, squalor of CA takes ca. 7 days(Baba and Shiraiwa 2012) and occurs in parallel with inhibition of active transport of bicarbonate(Bhatti and Colman 2008).

### Effects on lipid biosynthesis and fatty acid composition

Many research groups are presently affianced in the isolation of novel CO<sub>2</sub>-tolerant strains and investigations into their ability to build up storage lipids when sparged with high, or even pure, CO<sub>2</sub>(Yoo, Choi et al. 2012). In general, a moderate (2–5 %) increase in CO<sub>2</sub> stimulates both growth and lipid accumulation by microalgal cells(Muradyan, Klyachko-Gurvich et al. 2004). The availability of other carbon sources has a strong effect on lipid accumulation under elevated CO<sub>2</sub>. In particular, the possessions of high or low inorganic carbon concentrations (CO<sub>2</sub> and bicarbonate) on neutral lipid and starch accumulation were studied in the model alga *Chlamydomonas reinhardtii* during nitrogen depletion under photoautotrophic conditions(Gardner, Lohman et al. 2013). Under low CO<sub>2</sub> (0.04 %), carbon limitation resulted in reduced accumulation of both storage products - triacylglycerol (TAG) and starch; at 5 % CO<sub>2</sub>, the highest amount of TAG was produced, however under these circumstances rapid TAG accretion was followed by squalor (probably following acclimation to the elevated CO<sub>2</sub> level). Under high bicarbonate supplementation, the cell cycle was arrested and sustainable accumulation of both TAG and starch was recorded. In mixotrophic cultures (grown on an organic carbon source), raising CO<sub>2</sub> levels impaired the algae's capacity for import and assimilation of glycerol(Sforza, Cipriani et al. 2012).

Obviously, lipid (TAG in particular) biosynthesis represents a sink for the excess products of carbon fixation, which is especially important under hectic conditions causing termination of cell division(Solovchenko 2012).

One could further consider that the ability to channel the excess photosynthates to the biosynthesis of energy-rich compounds such as TAG may, in some situations, give to high-CO<sub>2</sub> tolerance of microalgae.

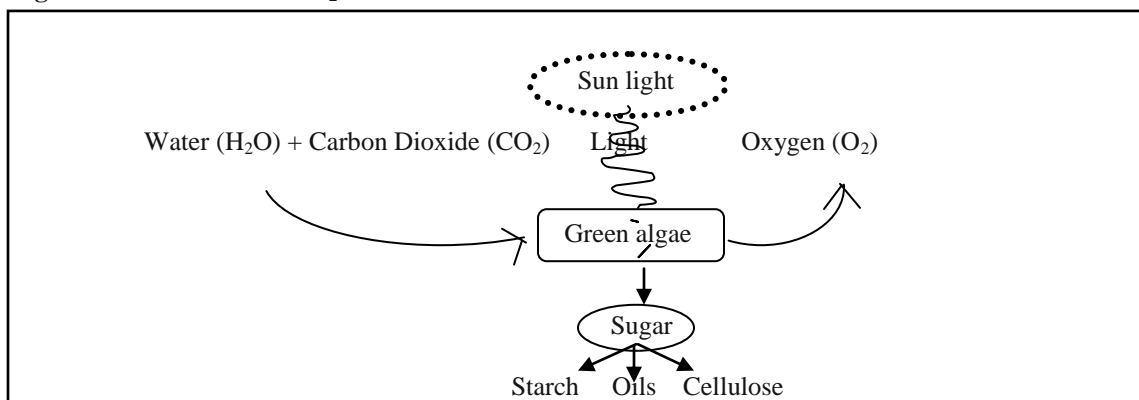
The enhance in CO<sub>2</sub> concentration in microalgae leads the growth and lipid accumulation in microalgae. *Chlamydomonas reinhardtii* showing the maximum triacylglycerol production.

### Conclusion

We are still some way from realizing the absolute potential offered by algal biodiesel. For the purpose of CO<sub>2</sub> sequestration, the use of microalgae is an exclusive technology. Life-cycle analyses suggest that using current methodologies the procedure is trivial in terms of positive energy balance and global warming potential. The actual process of photosynthesis, which can be utilized to fix carbon dioxide and produce useful by-products in a sustainable fashion, has been explained to be feasible and the design of a solar energized photo-bioreactor system for the function of carbon dioxide fixation was illustrated to be technically possible. CO<sub>2</sub> mitigation and biofuels production could be mutual in an economically feasible and environmentally sustainable approach. The possibility of this strategy could be further improved by fixing CO<sub>2</sub> from industrial exhaust gases such as flue gases and by integrating microalgal cultivation.

**Table.1-Range of significant attributes (CO<sub>2</sub> and temperature tolerance capacity) of commonly used microalgae**

Alga	CO <sub>2</sub> tolerance capacity (%)	Reference
Cyanidium caldarium	100	Seckbach et al., 1971
Eudorina sp. K17	20	Hanagata et al., 1992
Chlorella sp. K35	50	Hanagata et al., 1992
Scenedesmus sp. K34	80	Hanagata et al., 1992
Chlamydomonas sp. MGA 161	15	Miura et al., 1993
Nannochloropsis salina NANNP-2	10	Hamasaki et al., 1994
Chlorella sp. T-1	100	Maeda et al., 1995
Synechococcus elongates	60	Miyairi 1995
Monoraphidium minutum	13.6	Zeiler et al., 1995
Chlorella sp.	40	Sakai et al., 1995
Chlorella sp. UK001	40	Hirata et al., 1996
Euglena gracilis	45	Nakano et al., 1996
Chlorella vulgaris	15	Yun et al., 1997
Dunaliella tertiolecta	15	Nagase et al., 1998
Cyanidium sp.	100	Graham and Wilcox 2000
Chlorella sp.ZY-1	70	Yue and Chen 2005
Spirulina sp.	12	de Morais and Costa 2007a
Scenedesmus obliquus	18	de Morais and Costa 2007a
Chlorella kessleri	18	de Morais and Costa 2007b
Chlorococcum littorale	70	Ota et al., 2009
Chlorella minutissima	15	Sankar et al., 2011
Desmodemus sp.	100	Kativu et al., 2011

**Figure.1- Mechanism of CO<sub>2</sub> Fixation****Table.2- Lipid content in the dry biomass of various species of microalgae**

Species	Lipid content (% dry weight)
Anabaena cylindrical	4-7
Botyococcus braunii	25-80
Chlamydomonas reinhardtii	21
Chlorella vulgaris	14-22
Cryptocodium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella bioculata	8

Dunaliella primolecta	23
Dunaliella tertiolecta	35.6
Euglena gracilis	14–20
Hormidium sp.	38
Isochrysis sp.	25–33
Nannochloris sp.	30–50
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Pleurochrysis carterae	30–50
Porphyridium cruentum	9–14
Prymnesium parvum	22–38
Scenedesmus dimorphus	16–40
Scenedesmus obliquus	12–14
Schizochytrium sp.	50–77
Spirogyra sp.	11–21
Synechococcus sp.	11
Tetraselmis maculate	8
Tetraselmis sueica	15–23

### Future Prospects

To conclude, microalgae can securely be termed as the favorable system for CO<sub>2</sub> sequestration compared to forests and other vegetation. These should be chosen over others due to their possible of faster growth, high photosynthetic efficiency, environment friendly operation of effluent nutrients and flue gas, and providing a spectrum of value added mercantile products with no waste by-products. Continued progress of technologies to optimize the microalgae production, wastewater treatment, and biomass processing has the facility to make important contributions towards this object.

### References

- Baba, M. and Y. Shiraiwa (2012). "High-CO<sub>2</sub> response mechanisms in microalgae." *Advances in Photosynthesis-Fundamental Aspects*, In Tech, New York: 12-435.
- Beardall, J. and J. A. Raven (2013). *Limits to Phototrophic Growth in Dense Culture: CO<sub>2</sub> Supply and Light*. Algae for Biofuels and Energy, Springer: 91-97.
- Bhatti, S. and B. Colman (2008). "Inorganic carbon acquisition in some synurophyte algae." *Physiologia plantarum* **133**(1): 33-40.
- Change, I. P. O. C. (2007). "Climate change 2007: the physical science basis." *Agenda* **6**(07).
- Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology advances* **25**(3): 294-306.
- Chiu, S.-Y., C.-Y. Kao, C.-H. Chen, T.-C. Kuan, S.-C. Ong and C.-S. Lin (2008). "Reduction of CO<sub>2</sub> by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor." *Bioresource technology* **99**(9): 3389-3396.
- Chiu, S.-Y., C.-Y. Kao, M.-T. Tsai, S.-C. Ong, C.-H. Chen and C.-S. Lin (2009). "Lipid accumulation and CO<sub>2</sub> utilization of *Nannochloropsis oculata* in response to CO<sub>2</sub> aeration." *Bioresource technology* **100**(2): 833-838.
- de Morais, M. G. and J. A. V. Costa (2007). "Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide." *energy conversion and Management* **48**(7): 2169-2173.
- Fukuda, S.-y., I. Suzuki, T. Hama and Y. Shiraiwa (2011). "Compensatory response of the unicellular-calcifying alga *Emiliania huxleyi* (Coccolithophorales, Haptophyta) to ocean acidification." *Journal of oceanography* **67**(1): 17-25.
- Gardner, R. D., E. Lohman, R. Gerlach, K. E. Cooksey and B. M. Peyton (2013). "Comparison of CO<sub>2</sub> and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*." *Biotechnology and Bioengineering* **110**(1): 87-96.

- Hempel, N., I. Petrick and F. Behrendt (2012). "Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production." *Journal of Applied Phycology* **24**(6): 1407-1418.
- Huertas, I. E., B. Colman, G. S. Espie and L. M. Lubian (2000). "Active transport of CO<sub>2</sub> by three species of marine microalgae." *Journal of Phycology* **36**(2): 314-320.
- Miyachi, S., I. Iwasaki and Y. Shiraiwa (2003). "Historical perspective on microalgal and cyanobacterial acclimation to low-and extremely high-CO<sub>2</sub> conditions." *Photosynthesis research* **77**(2-3): 139-153.
- Muradyan, E., G. Klyachko-Gurvich, L. Tsoglin, T. Sergeyenko and N. Pronina (2004). "Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO<sub>2</sub> concentration." *Russian journal of Plant physiology* **51**(1): 53-62.
- Ono, E. and J. L. Cuello (2003). Selection of optimal microalgae species for CO<sub>2</sub> sequestration. Second National Conference on Carbon Sequestration.[Online].
- Ota, M., Y. Kato, H. Watanabe, M. Watanabe, Y. Sato, R. L. Smith Jr and H. Inomata (2009). "Fatty acid production from a highly CO<sub>2</sub> tolerant alga, *Chlorococcum littorale*, in the presence of inorganic carbon and nitrate." *Bioresource Technology* **100**(21): 5237-5242.
- Pesheva, I., M. Kodama, M. L. Dionisio-Sese and S. Miyachi (1994). "Changes in photosynthetic characteristics induced by transferring air-grown cells of *Chlorococcum littorale* to high-CO<sub>2</sub> conditions." *Plant and cell physiology* **35**(3): 379-387.
- Ramanan, R., N. Vinayagamorthy, S. D. Sivanesan, K. Kannan and T. Chakrabarti (2012). "Influence of CO<sub>2</sub> concentration on carbon concentrating mechanisms in cyanobacteria and green algae: a proteomic approach." *Algae* **27**(4): 295-301.
- Raven, J. A., M. Giordano, J. Beardall and S. C. Maberly (2012). "Algal evolution in relation to atmospheric CO<sub>2</sub>: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles." *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**(1588): 493-507.
- Rienmüller, F., I. Dreyer, G. Schönknecht, A. Schulz, K. Schumacher, R. Nagy, E. Martinoia, I. Marten and R. Hedrich (2012). "Luminal and cytosolic pH feedback on proton pump activity and ATP affinity of V-type ATPase from *Arabidopsis*." *Journal of Biological Chemistry* **287**(12): 8986-8993.
- Rubio, F. C., F. G. Camacho, J. Sevilla, Y. Chisti and E. M. Grima (2003). "A mechanistic model of photosynthesis in microalgae." *Biotechnology and bioengineering* **81**(4): 459-473.
- Satoh, A., N. Kurano, H. Senger and S. Miyachi (2002). "Regulation of energy balance in photosystems in response to changes in CO<sub>2</sub> concentrations and light intensities during growth in extremely-high-CO<sub>2</sub>-tolerant green microalgae." *Plant and cell physiology* **43**(4): 440-451.
- Sforza, E., R. Cipriani, T. Morosinotto, A. Bertucco and G. M. Giacometti (2012). "Excess CO<sub>2</sub> supply inhibits mixotrophic growth of *Chlorella protothecoides* and *Nannochloropsis salina*." *Bioresource technology* **104**: 523-529.
- Solovchenko, A. (2012). "Physiological role of neutral lipid accumulation in eukaryotic microalgae under stresses." *Russian Journal of Plant Physiology* **59**(2): 167-176.
- Sudhakar, K. and M. Premalatha (2012). "Micro-algal technology for sustainable energy production: state of the art." *Journal of Sustainable Energy & Environment* **3**: 59-62.
- Sydney, E. B., W. Sturm, J. C. de Carvalho, V. Thomaz-Soccol, C. Larroche, A. Pandey and C. R. Soccol (2010). "Potential carbon dioxide fixation by industrially important microalgae." *Bioresource technology* **101**(15): 5892-5896.
- Wang, B., Y. Li, N. Wu and C. Q. Lan (2008). "CO<sub>2</sub> bio-mitigation using microalgae." *Applied Microbiology and Biotechnology* **79**(5): 707-718.
- Yoo, C., G.-G. Choi, S.-C. Kim and H.-M. Oh (2012). "*Ettlia* sp. YC01 showing high growth rate and lipid content under high CO<sub>2</sub>." *Bioresource technology*.
- Zarco-Tejada, P., A. Catalina, M. González and P. Martín (2013). "Relationships between net photosynthesis and steady-state chlorophyll fluorescence retrieved from airborne hyperspectral imagery." *Remote Sensing of Environment* **136**: 247-258.