International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue | A21 | July 2023
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

Chlorella: Abundance, Applications, and Prospects of CO_2 Fixation - A North East India Perspective

Dhar Rhea*, Devi Moirangthem Bidyaswori, and Paul Dibyendu

Department of Environmental Studies, North-Eastern Hill University, Shillong-793022, Meghalaya, India
*Corresponding author: rheadhar2010@gmail.com

Manuscript details:

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Dhar Rhea, Devi Moirangthem Bidyaswori, Paul Dibyendu (2023) *Chlorella*: Abundance, Applications, and Prospects of CO_2 Fixation - A North East India Perspective, *Int. J. of. Life Sciences*, Special Issue – A 21 : 7-16.

Article published in Special Issue on "*Environmental Pollution: Challenges and Strategies for Mitigation*"

ABSTRACT

Chlorella, a green microalga has drawn a lot of interest because of its rapid growth, rich nutritional profile, and ability to treat wastewater and fix carbon dioxide. The review emphasizes the unique habitats where Chlorella has been reported in North East India and successful cultivation techniques demonstrating its biochemical and phycoremediation properties and lipid productivity. Chlorella has shown enormous promise in carbon dioxide fixation, environmental restoration, and renewable energy, and the factors that play a significant role in Chlorella's capacity to absorb carbon dioxide are highlighted here. Studies conducted from NE India revealed the region's significant reservoir of Chlorella and highlight the potential of this region as a promising hub for future exploration and utilization to produce sustainable energy and mitigate the increase in carbon dioxide concentrations.

Keywords: *Chlorella*, CO_2 sequestration, North East India, Sustainable energy.

INTRODUCTION

The rapid increase in carbon dioxide levels post the Industrial Revolution from 280 ppm in the 1700s to 421 ppm in May 2022, as reported by the NOAA's Mauna Loa Atmospheric Baseline Observatory (USA) accounts for 50% higher carbon dioxide levels than in the pre-industrial period. Many anthropogenic activities, including using fossil fuels for electricity generation and transportation, industries, deforestation, etc., have caused this exponential increase, leading to global warming. India's growing economy is one of the major reasons for the increase in energy consumption. Energy consumption, mostly due to the use of fossil fuels, has led to skyrocketing increase in carbon dioxide emissions in the last ten years (Shaw and Mukherjee, 2022). The first-ever initiative at an international level to address climate change was undertaken at the UNFCCC's Kyoto Protocol in 1997, wherein developed countries were legally bound to reduce carbon dioxide emissions to minimize the adverse effects of climate change (Singh and Ahluwalia, 2013). Scientists have also studied that the emission of carbon dioxide into the atmosphere is much more than what the natural sinks can remove, and to counteract this problem, various carbon dioxide sequestration methods are being researched. Amongst the physical, chemical, and biological methods of sequestration, the physical and chemical methods are not greatly preferred owing to high transportation, capturing, storage costs, and environmental safety concerns (Kumar *et al.*, 2011, Singh and Dhar, 2019). The biological sequestration method is a better alternative to the physical and chemical methods. Algal carbon dioxide sequestration is a promising technology due to its ability to fix carbon dioxide 10-50 times more than terrestrial plants, high photosynthetic efficiency, and faster growth than higher plants, biofuel, and other by-product generation, and flexible cultivation options (Basu *et al.*, 2014, Batista *et al.*, 2015, Bhola *et al.*, 2014, Cheah *et al.*, 2015, Costa *et al.*, 2000).

To date, various algae like *Chlorella*, *Chlamydomonas* (Barati *et al.*, 2022), *Coccomyxa* (Zhu *et al.*, 2017), *Microcystis*, *Nannocchloropsis* (Bhola *et al.*, 2014), *Oscillatoria* (Anguselvi *et al.*, 2019), *Pseudokirchneriella*, *Scenedesmus* (Tang *et al.*, 2011), *Spirulina* (Priscilla Centeno da Rosa *et al.*, 2011), *Synechocytis*, etc., have been studied for their carbon dioxide mitigation properties. *Chlorella* is considered a promising species for bioremediation and carbon dioxide fixation (Onyeaka *et al.*, 2021) and is used as a model for various carbon assimilation studies (Masojídek and Torzillo, 2014). *Chlorella* can grow under conditions up to 40% v/v carbon dioxide concentration (Brennan and Owende, 2010) with carbon capture efficiency up to 92% - 93.7% (Song *et al.*, 2021, Yang *et al.*, 2020) and carbon dioxide fixation rate up to 2.22 g/L/day (Cheah *et al.*, 2015). *Chlorella* can utilize carbon dioxide and NO_x and SO_x present in flue gas efficiently without affecting its biomass production (Singh and Singh, 2015) and decrease the concentration of NO_x and SO_x efficiently (Kao et al., 2014). Higher NO_x content aids in faster growth (Kao *et al.*, 2014), and dissolved NOx helps to provide a source of nitrogen for algal growth (Chen and Wang, 2020). Lee *et al.* (2002) reported up to 60.5% relative growth rate when *Chlorella* species KR-1 was studied under flue gas containing 60 ppm of SO_x. *Chlorella* species have also been used for the bioremediation and cost-effective treatment of wastewater (Pooja *et al.*, 2022) and aid in the reduction of nutrients and organic matter and improved quality for safe disposal of the effluent. These studies suggest that *Chlorella* has considerable promise as a bioremediation solution not only for wastewater but also for the fixation of carbon dioxide and other greenhouse gases.

Carbon dioxide sequestration efficiency in *Chlorella*

The factors that play a key role in influencing the carbon dioxide sequestration efficiency in *Chlorella* include;

Temperature

Temperature influences algae growth rate, cell size, carbon dioxide solubility, biomass output, and lipid content. Temperature below the optimal limit of microalgae growth alters the kinetics of cellular enzymatic activities. Exceeding the limiting temperature affects photosynthesis efficiency, resulting in a fall in biomass production, energy conversion efficiency (Serra-Maia, 2016), and even cell death. While the ideal temperature is known to be within the range of 20°C - 30°C, some *Chlorella* strains are known to tolerate temperatures up to 40°C - 45°C (Masojídek and Torzillo, 2014, Ziganshina *et al.*, 2022). Increasing temperature increases the cell's metabolic processes, which directly affects the photosynthesis rate and the carbon dioxide fixation property. Respiration rate and membrane fluidity are also affected by fluctuations in temperature. The range of optimal temperatures which prove to be beneficial for *Chlorella* growth and increased biomass production are 25°C, 27°C, 28°C and 30°C (Aleya *et al.*, 2011, Han *et al.*, 2013, Singh and Singh, 2015, Yang *et al.*, 2010, Yun *et al.*, 2001).

pH

Maintaining a suitable range of pH is essential for solubility and availability of nutrients and carbon dioxide, biochemical reactions, and cellular metabolism that promote growth (Juneja *et al.*, 2013). Some algae can tolerate and grow at higher pH, like 9, and even low pH, like 4, but most algae grow at a neutral pH of 6 - 8. pH affects nutrient availability, physiological processes, and enzyme activity which affects growth (Gao *et al.*, 2022, Kuo *et al.*, 2017, Sakarika and Kornaros, 2016) and carbon dioxide availability. Flue/ exhaust gas generally contains other components like NO_x and SO_x in addition to carbon dioxide, which can lower the pH of the algal medium and affect growth. In such cases, pH adjustment is necessary to ensure proper growth and photosynthetic efficiency. It has been studied that the optimal pH for *Chlorella*'s growth is around 7 (Wang *et al.*, 2010), 10-10.5 (Gong *et al.*, 2014), 7-8 (Sarker and Salam, 2019, Yu *et al.*, 2022).

Light intensity

Light intensity is one of the most important factors for microalgal photosynthesis, growth, and carbon dioxide fixation (Morales *et al.*, 2017). It also influences the composition and output of biomass generation (Li *et al.*, 2012). In the process of photosynthesis, light intensity plays an important role in the light-dependent stage that is used to fix carbon dioxide. An optimal range of light is necessary because low light intensity leads to inhibition of microalgae development (Lee *et al.*, 2015) and low biomass production and high light intensity lead to cell stress. The recommended light intensity for the growth of *Chlorella* species ranges from 50 to 300 μmol/m^2/sec of photosynthetic active radiation. Effects of different light intensities were studied which suggest the light intensity of 80 μmol/m^2/sec of warm white light (Khalili *et al.*, 2015), 16/8 h light: dark of intensity 8000 lux (approx. 107.9 μmol/m^2/sec) (Gunawan *et al.*, 2018), 10,000 lux (Febrieni *et al.*, 2020), and 6400 lux (Lamadi *et al.*, 2022) light intensity for 24 hours, 150 μmol/m^2/sec at 16/8 h light: dark photoperiod (Gao *et al.*, 2022) yield the maximum growth.

Carbon dioxide concentration

Carbon dioxide is actively taken up by algae through stomata, and carbon dioxide diffuses across the chloroplast membranes and reaches the site of carbon fixation. Algae possess a greater ability to fix carbon dioxide and most algae show luxurious growth between 2% - 5% carbon dioxide level. During photosynthesis, algae release oxygen in the surrounding environment, replenishing the oxygen levels. Several studies have shown that increasing carbon dioxide levels improves growth, however, the concentration for maximum growth rate varies (Thomas *et al.*, 2016). The concentration of carbon dioxide in the growth medium directly affects the rate of carbon dioxide uptake and fixation in *Chlorella*. *Chlorella* can grow well at carbon dioxide concentrations ranging from 3% - 40% (Barahoei *et al.*, 2020, Chen *et al.*, 2014, Clément-Larosière *et al.*, 2014, Shabani *et al.*, 2016). Microalgae fed with flue gases contain NO_x and SO_x in addition to carbon dioxide which helps by increasing carbon bio-fixation and biomass productivity (Singh and Dhar, 2019).

Cultivation techniques

The most common microalgae cultivation methods include the open cultivation systems such as raceway ponds and tanks, and closed cultivation systems which include different types of photobioreactors. Raceway ponds require low-cost and low-energy inputs but are prone to contamination, require a large set-up area, and have low productivity as compared to photobioreactors. Whereas, photobioreactors are expensive but, have higher biomass productivity, require less set-up area, and have low contamination risks (Jorquera *et al.*, 2010).

***Chlorella* and North East India**

North East India is one of the mega biodiversity-rich areas in the world, and its unique location composed of distinct habitats supports diverse algal communities. Known for having high biodiversity, this region of India has received little research (Ratha *et al.*, 2012). The region's high rainfall, moderate temperature, stretches of tropical rain, and tropical deciduous forests are home to a wide range of endemic flora and fauna, creating a variety of microenvironments for algal colonization and excellent for the growth of microalgae. Algae occupies the lowest level in the food chain, and the presence of various rivers, lakes, beels, and waterfalls in this region (Medhi and Kalita, 2020) houses a plethora of algal communities. The microalga *Chlorella* is ubiquitous in both terrestrial and aquatic environments (Safi *et al.*, 2014, Teoh *et al.*, 2004) owing to its simple morphological structure, small cell size, resilience to environmental stress (Hodač *et al.*, 2016), adaptability, high reproduction capability, and flexible habitat range. *Chlorella* species in the NE region have been reported in various habitats ranging from freshwater bodies to lakes and beels, wetlands, sacred groves, forest soils, rock surfaces, and even tree barks. Even though North East India is rich in microalgal diversity, there are few reports on algal abundance and taxonomic publications are restricted to the study of a few aquatic environments of the region only (Bora *et al.*, 2016). Here, an attempt has been made to consolidate existing literature and reports on the occurrence of *Chlorella* species in the region, as well as the work that has been done and the potential that it holds for future carbon dioxide sequestration studies. With respect to NE India, *Chlorella* species that have been reported in the region are given in Table 1:

Table 1: *Chlorella* and its habitat reported from NE India

	Algae	**Authors**	**Habitat**
Assam	*Chlorella* species	Devgoswami *et al.* (2011)	-
	Chlorella sorokiniana FC6 IITG	Kumar *et al.* (2014)	Freshwater pond
	Chlorella species FC2 IITG	Muthuraj *et al.* (2014)	Freshwater pond
	Chlorella sorokiniana (NEIST -BT2)	Sehgal *et al.* (2019)	Freshwater body (Upper Brahmaputra valley)
	Chlorella vulgaris BS1	Das and Deka (2019)	Oil field formation water
	Chlorella homosphaera	Sharma *et al.* (2020)	-
	Chlorella minutitissima (Fott *et* Novakova)	Yasmin *et al.* (2015)	River, beel, ditch (Kaziranga National Park)
	Chlorella species	Lahan *et al.* (2012)	-
	Chlorella species	Acharjee *et al.* (2021)	Floodplain wetland (Chatla lake/ *Chatla Haor*)
	Chlorella ellipsoidea	Borah *et al.* (2020)	Domestic sewage water
	Chlorella sp. MP-1	Phukan *et al.* (2011)	Manmade lake (Joysagar)
	Chlorella sp. DRLMA3	Kaur *et al.* (2012)	Pond, paddy fields
	Chlorella species	Goswami and Kalita (2012)	Factory wastewater, ponds (Chandrapur area)
	Chlorella ellipsoidea	Purkayastha *et al.* (2017)	Freshwater lake (Padum Pukhuri, Tezpur)
	Chlorella chlorelloides *Chlorella vulgaris*	Baruah *et al.* (2020)	Wetland (Deepor Beel)
Tripura	*Chlorella* sp. NITAAP009 *Chlorella* sp. NITAAP011	Ghosh *et al.* (2017)	Lake, moist soil
	Chlorella thermophila	Sarkar *et al.* (2020)	Lake (Neer Mahal), moist soil (Jirania)
	Chlorella species	Bharati *et al.* (2020)	Wetland (Rudrasagar lake)
Meghalaya	*Chlorella vulgaris* Oettli	Ray and Das (2021)	-
	Chlorella vulgaris Beijernick *Chlorella saccharophila* (W. Kruger) Migula *Chlorella* species *Chlorella* species 1	Kharkongor and Ramanujam (2014)	Tree barks (undisturbed sacred grove, mixed plantation, open disturbed forest)
	Chlorella sp. NC-MKM	Mandal and Chaurasia (2018)	Waterfall (Nohkalikai)
	Chlorella vulgaris	Barman *et al.* (2015)	Wetlands (West Garo Hills)
	Chlorella saccharophila (Krüger) Migula *Chlorella vulgaris* Beyerrinck (Beijerinck)	Dirborne and Ramanujam (2017)	Soil (sacred grove, pine forest)
	Chlorella sp. MIC-G12	Ratha *et al.* (2012)	Lake
Arunachal Pradesh	*Chlorella* species	Ganie *et al.* (2018)	Rivers (Dirang chu and Tenga)
	Chlorella species	Awasthi (2021)	Rice-fish field

Application of *Chlorella* species

The application of *Chlorella* species studied in North East India to date has been the subject of much research, paving the way for their potential uses some of which are:

Lipid production

Chlorella is extensively studied for lipid production due to its easier cultivation and faster growth (Lv *et al.*, 2010). *Chlorella ellipsoidea*, an isolate from Padum Pukhuri, Tezpur, was examined to have lipid productivity of 1184 mg/L. A high amount of protein and carbohydrate was also reported from this species (Purkayastha *et al.*, 2017). Different modes of cultivation have been studied for the generation of maximum biomass and lipid productivity. *Chlorella* species FC2 IITG reported maximum total lipid productivity of 50.42 mg/L/day and maximum biomass productivity of 114 mg/L/day under mixotrophic and photoautotrophic cultivation conditions respectively (Muthuraj *et al.*, 2014). Lipid productivity of 550 mg/l/day and biomass productivity of 1930 mg/L/day was reported (Kumar *et al.*, 2014) when *Chlorella* species FC6 IITG was studied under fed-batch conditions with glucose and acetate as growth inducers. The versatility of *Chlorella* species in adapting to different cultivation conditions helps to optimize productivity for lipid production. Sehgal *et al.* (2019) studied seven different native microalgae and found *Chlorella sorokiniana* NEIST BT-2 to be the most promising in terms of lipid productivity (107 mg/L/day) and biomass production (2090 mg/L). Sharma *et al.* (2020) reported 31% lipid content in *Chlorella homosphaera* when cultivated in 10% goat droppings. Organic wastes such as goat droppings and cow dung manure are sustainable nutrient management strategies studied for microalgae cultivation. *Chlorella* sp. MP-1 and another *Chlorella* species (isolated from wastewater of a brick kiln) reported 28.82% and 25% lipid content respectively (Goswami and Kalita, 2012, Phukan *et al.*, 2011). An isolate from ponds and paddy fields, *Chlorella* sp. DRLMA3 was examined to find 20% lipid content (Kaur *et al.*, 2012) as paddy/ rice fields are nutrient-rich habitats for phytoplanktons. Mandal and Chaurasia (2018) reported a 92.8% increase in lipid content when *Chlorella* sp. NC-MKM isolated from a waterfall in Meghalaya was subjected to nitrogen depletion conditions. Lipid synthesis may be stimulated in nutrient-depleted situations, making it a reliable and long-lasting source of renewable energy. The studies based on the lipid production of noble isolates from the NE region increase our understanding of microalgae cultivation methods, nutrient management, and potential *Chlorella* applications in the bioenergy and biotechnology sectors. Further in-depth study of these strains will lead to the development of reliable and efficient microalgae-based systems for lipid synthesis and carbon dioxide sequestration in these specific locations.

Biochemical analysis

Various factors affect the chemical composition of algae which in turn, affects the biofuel productivity and ability to fix carbon dioxide. Amongst the indigenously isolated *Chlorella* species, Ghosh *et al.* (2017) reported *Chlorella* sp. NITAAP009 and NITAAP011 with high protein and high chlorophyll content and 12% lipid content respectively. *Chlorella thermophila* was found to have 2.7 times more chlorophyll content when extracted from wet instead of dry biomass (Sarkar *et al.*, 2020). The biochemical analysis provides insight into the metabolic pathways involved in carbon dioxide fixation, which helps in optimizing the efficiency of carbon dioxide fixation in algae.

Phycoremediation

Phycoremediation/ bioremediation using algae is a potential approach for integrated nutrient removal and sequestration, with subsequent biomass generation to manage environmental pollution (Kumar *et al.*, 2018). Das and Deka (2019) isolated *Chlorella vulgaris* BS-1 from oil field formation water which demonstrated 1.76 mg/L/day biomass productivity and removed 98% petroleum hydrocarbon, 75% COD, and 100% sulphate from the formation water. The reduction in cost of wastewater treatment, and production of valuable biomass when *Chlorella* is used for nutrient recovery has gained more interest in recent times (Li *et al.*, 2021, Sánchez-Zurano *et al.*, 2021). In addition to the detection of hazardous substances and removing nutrients from wastewater, it also eliminates waste carbon dioxide, which lowers greenhouse gas emissions. The procedure is economical and environmentally benign, and the algal biomass with high nutritional value is suitable for use as aquaculture feed and biofertilizer (Gani *et al.*, 2015).

CONCLUSION

The potential use of microalgae in addressing various environmental challenges has recently gained attention on a global scale. North East India, being a major centre of biodiversity is home to a diverse algal population, especially *Chlorella*, but it has not been properly explored (Bora *et al.*, 2011). *Chlorella* species has been established as a potential microalga to produce biomass, lipids, proteins, carbohydrates, and pigments (Balasubramaniam *et al.*, 2021). The climatic condition of this region is highly favourable for the growth of microalgae and provides ample scope for phycological research for the identification of potential candidates for various biotechnological applications (Bora *et al.*, 2016). The diversity in the habitats of *Chlorella* species reported from this region varies largely and has shown promising results when grown under various cultivation conditions over a range of temperatures. This can be utilized to create effective wastewater treatment methods, renewable feedstock for the manufacture of biodiesel, and carbon dioxide fixation studies.

The low-lying rice fields of this region are nutrient-rich habitats for phytoplanktons, brick kiln/ factory wastewater and ponds where flue gas from kilns/ factories are directly transported are also nutrient-rich habitats for microalgae. With the right technology, ponds can be built for the mass cultivation of microalgae in such a way that allows different flue gases to be delivered directly to the pond system for sustainable energy development. This will improve the environment and economy by reducing harmful greenhouse gases, producing ecologically beneficial biofuel, and treating industrial effluent (Goswami and Kalita, 2012). Strains from extremely low-temperature locations grown under nutrient-depleted conditions highlight the importance of these habitats as selecting criteria for exploratory study for the future (Ratha *et al.*, 2012) and help to shed light on how algae adapt to stressful environments and proliferate and maintain their ability to fix carbon dioxide efficiently. This may be further investigated to examine the ability to sequester carbon dioxide and other greenhouse gases efficiently. The biochemical analysis offers insightful information on the elements that affect carbon dioxide fixation in algae, such as their nutritional makeup, metabolic processes, stress responses, bioproduct potential, taxonomic identification, and growth optimization. Understanding and adjusting these variables can help algae-based systems become more effective at fixing carbon dioxide and advance their usage as long-term solutions for carbon sequestration and greenhouse gas emission reduction.

The microalga *Chlorella*, which is widely distributed in NE India, has a lot of potential for use in various fields. Particularly because of its high lipid productivity and ability to remediate wastewater, it offers promising opportunities for addressing the issues related to green fuel production and nutrient removal. In addition to this, the photosynthetic abilities of *Chlorella* can be applied to study cutting-edge methods for capturing and sequestering carbon dioxide, lowering greenhouse gases. To maintain future energy security and to mitigate the ever-rising problem of increase in temperature, it is imperative to make use of the North East's rich algal resource and use it sustainably as a feedstock for the manufacture of biodiesel and to mitigate the effects of global warming.

Acknowledgments
The authors gratefully acknowledge the financial support of UGC-SVSGC and NEHU Non-NET fellowships.

Conflicts of interest: The authors stated that no conflicts of interest.

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