Chapter 1

GENERAL INTRODUCTION
Introduction

1.1 Algal blooms
Algae are microscopic single and multiple-celled aquatic plants containing chlorophyll and other photosynthetic pigments. Most of the individual algal cells can only be seen under the microscope. When dense algal populations develop they turn water into a green or greenish brown color referred to as “bloom”. When populations of algal cells multiply giving color to the pond, it is referred as algal bloom. Blooms are simply high concentrations of algal cells that give the water a “pea soup” appearance. Algal blooms developed when the water bodies are enriched with organic or inorganic fertilizer either naturally or by any anthropogenic sources. The natural and artificial fertilizers provide principal chemical nutrients needed for algal growth and reproduction. Besides principal nutrients nitrogen (N), phosphorus (P) and potassium (K), penetration of light, temperature, salinity, pH play important role for growth and density of algal
bloom (Mahar et al., 2000). The nutrients may be contributed by detritus of algae and other plant species. Phosphates along with other salts are the prime contributors for degradation of water quality due to the formation of algal blooms leading to eutrophication (Kousar and Puttaiah, 2008). Infact algal blooms are taken as the symptom of eutrophication. This usually occurs because of the progressive eutrophication of water bodies. Eutrophication is a natural process of accumulation of nutrients leading to increased aquatic plant growth in the water body (Nixon, 1995). The term ‘eutrophic’ is a Greek word which means ‘well fed’. When nitrogen and phosphorus concentration increases, the process of eutrophication sets in and primary production is accelerated (Harper, 1992). Human activities contributing fertilizers and other high nutrient wastes can speed up the process leading to excessive algal blooms and deterioration of water quality. Algal blooms appear as a consequence of nutrient inputs from the watershed. According to World Health Organization (WHO, 2009) an algal bloom is a rapid increase in the population of algae in the aquatic system. Algae can multiply quickly in water bodies with an over abundance of nitrogen and phosphorus, particularly when the water is warm and the weather is calm. Only one or few species of algae are normally involved in blooms formation. Blue green and green algae are the main bloom forming organism. According to Anderson (1997) a great variety of algal species can be found in water samples with algal bloom some of which are harmful. There is growing evidence that bloom initiating mechanisms are diverse and are controlled by several environmental and physiological factors (Paerl, 1996, Hyenstrand et al., 1998, Oliver and Ganf, 2000). Algal bloom in aquatic ecosystem has been known since the time of Captain Cook who first recorded an algal bloom during his voyage in 1770. Under certain conditions, floating algal cells at the surface of water form a layer known as “scum” Such scums are usually formed during still weather after a period of warm and windy conditions. This layer are pushed to one side by the wind forming a thick mass of algae. Scums appear in a variety of colors like yellow, green, bluish green or red. Exposure to intense sunlight kill algae turning some cells white so that scums may also develop mottled colors. Red euglenoids are responsible for red scums in freshwater ponds.
Fig.1.1 Schematic representation of bloom formation (source: Russian Academy of Science)

1.2 Beneficial and Harmful aspects of algal blooms

Algal blooms are favored for a number of reasons as it increases the primary productivity of pond ecosystem. As microscopic ‘grass’, the bloom may serve as food for microscopic animals like zooplanktons that forms the base of the food chain supporting larger forms of life such as insects and fishes. Increasing the base of the food chain enhances the total productivity of the pond. The blooms can also control initial growth of larger aquatic plants such as macrophytes by increasing turbidity, blocking sunlight and reducing the young plant’s photosynthesis. The
blooms of algae can grow throughout the year with an annual biomass productivity that surpasses that of terrestrial plants by about tenfold times (Hu, et al., 2008). However, increase in intensity and frequency algal blooms may cause community concern for health polluting the environment. Ecological impacts apart, there are social and economic aspects related to it. It disturbs the natural balance of plant and animal ecosystems in the water body. It degrades recreation, conservation and scenic values and also interferes with economic uses such as fishery activity. Excessive growth of algae can become fatal for fishes. When the algae in the bloom dies the decay process consume all the available oxygen in the water which suffocate aquatic life forms like fish, crabs, insects etc. Blooms of benthic or planktonic macroalgae are of major ecological significance such as the displacement of indigenous species, habitat destruction, oxygen depletion, including alteration of biogeochemical cycles (ECOHAB, 2005). The World Health Organization (WHO) standard indicate that when the population of algal cells exceeds 100,000 cells/ml, or the equivalent of about 24 million algal cells in an 8-ounce cup of water, blooms causes severe problems. States may have different standards and health agencies may test for specific toxins as well. Blooms cause problem in the summer months and are more frequent in times of drought. For instance, bloom caused by green algae consume nutrients and excrete soluble oxygen inhibiting the development of blue green algae bloom (Mun, 2003). Certain blooms are even harmful to other organism and termed as harmful algal blooms (HABs). They deplete oxygen levels in the aquatic ecosystem and also reach mechanical damage to other organism. The toxins released are dangerous to animals including human beings. More than 300 species of microalgae are documented to form mass occurrence and nearly \( \frac{1}{4} \) th of these species are known to be toxic. Among Euglenoids, *E. sanguinea* has been identified as a dominant HAB present in a number of fish kill events (Zimba, et al., 2004). Toxic bloom events have occurred in a number of foreign countries like North Carolina (2 additional events), South Carolina (1 event), Texas (3 events), Arkansas (1 event), and Mississippi (4 events). But such type of events have not yet been reported from India, neither large scale fish mortality is recorded from North-East India or for that matter from Cachar district.

### 1.3 Assessment of algal blooms

Algal blooms are monitored using ‘biomass’ measurements. Chlorophyll concentration are also used for monitoring algal blooms. Bloom density in pond water is measured using an instrument known
as Secchi disk. A Secchi disk is a circular flat disk, typically 8 inches in diameter, made from wood or plastic and painted white or with black and white quadrants. The disk is mounted on a yardstick. The cut-off bottom of a plastic bleach bottle works well and the yardstick can be fastened to handle. To use the disk, it is inserted upright in the water and lower until the circular disk just disappears from view, the water depth on the yardstick is read. The disk is slowly raised until it can be seen again and the depth is read again. The average of the two readings is the “Secchi depth.” Infertile water bodies should have Secchi depths of greater than 3 feet, and in these waters, Secchi disks must be weighted and suspended from calibrated ropes in order to obtain accurate readings. Blooms are considered to be too dense when the Secchi depth is > 12 inches.

Green algae (chlorophyceae) are the strain most favored by researchers. However, it tend to produce starches instead of lipids and require nitrogen to grow. They have very high growth rates at 30°C and at high light levels in aqueous solution. All algae are primarily made up of proteins, carbohydrates, fats, and nucleic acids in varying proportions and are rich in carbonaceous compounds. The relative distribution can vary with the type of algae, some types of algae are made up of up to 40% fatty acids based on their overall mass. This fatty acid may be extracted and converted into biofuel (Gross, 2009). Algal blooms that are grown at different light intensities show remarkable changes in their gross chemical composition, pigment content and photosynthetic activity (Guschina and Harwood, 2005).

1.4 *Euglena* bloom

*Euglena* is the simplest, single, self-sufficient cell which relies only on sunlight, carbon dioxide and minerals from the water. *Euglena*, found in freshwater environments is a popular flagellated laboratory test microorganism. They are present in high numbers in nutrient rich waters. Other *Euglena* species can found in soil and also in marsh lands as thick mats (Wolken, 1961, Pennak, 1979). The taxonomy of this unique organism is debated for many years. It represents one of the earliest derived eukaryotic protist with both plant and animal like features. Typified as a member of the algal division Euglenophyta it functioning both as a photoautotroph and chemoheterotroph. It requires H, C, N, O, Mg, P, S, Cl, K, Ca, Mn, Co, Zn, and some other elements at very low levels (Wolken, 1961). It produces oxygen at a high rate; reduce carbon dioxide and breaks down organic matter (Chae, *et al.*, 2006).
Euglena possess elongated cell with one nucleus that contain pigmented chloroplast which helps in photosynthesis, a contractile vacuole for excretion, an eye spot to spot sunlight and flagella for movement. *Euglena* undergo asexual mode of reproduction multiplying through cell division. It lives in fresh and brackish water rich in organic matter often appearing as green or red “blooms” in pond or lakes. Several species produce breathing vesicles that can resist drying. It ingests food into the gullet and basically serve as a food source for fish and other marine critters. *Euglena* differs from the majority of the other flagellates and shows an approach to the true algae (Fedun, 2012). It is the most primitive phylum containing chloroplast and their thylakoids differ from those of higher plants because it is unstacked (Doege et al., 2000).

*Euglena* assemblages are known to be widely distributed in higher eutrophicated shallow ponds at elevated temperature (Wild et al., 1995). Thick bloom of *Euglena* is detrimental to the growth of phytoplankton as it inhibit light penetration in the water body and also use nutrients from the water for their growth and development (Rahman et al., 2007). *Euglena gracilis* is a free living protist that tolerates high concentrations of different heavy-metals (Navarro et al., 1997; Devars et al., 2000; Mendoza-Coztatl et al., 2002). *E. caudata* and *E. viridis* shows high biomass concentration throughout the year in freshwater ecosystem (Kim and Boo, 1996). In the day time when the sunlight is intense the calm water become reddish which is caused due to the presence of a pigment called carotenoids that accumulated in the *Euglena* cells. The production of red pigment is believed to be due to response to intense sunlight which protects the chlorophyll from degradation (Rhodes, 2010). Spread over 44 genera more than 900 species of *Euglena* have been discovered so far, (Kanika Khara, 2010).

*Euglena* is very common in Cachar district of Assam and inhabits in fresh water environments. They are present in high numbers in nutrient rich waters. Other *Euglena* species can be found in soil, freshwater environments. *Euglena* can exist in marsh lands as thick mats. It can produce oxygen at a high rate and can significantly reduce carbon dioxide. It also breaks down organic matter. *Euglena* confused researchers because it is green like a plant and carried out photosynthesis. It is considered as plant due to the presence of photosynthetic pigments but like plant *Euglena* does not have cellulose cell wall. It is an ideal laboratory organism to study different aspect because of its huge potentiality, adaptive abilities and metabolic flexibility. Euglenoids are used as biological indicators of water pollution (Kim and Boo, 1996). Healthy
cells liberate polysaccharides to prevent the cell from injury, dead or decaying. The exponential growth of *Euglena* blooms throughout the year may be one of the reasons for the high cellular carbohydrates concentration (Shankar et al., 2000). As compared to other unicellular green algae, *Chlorella* and *Scenedesmus* (Ghose, 2011), *Euglena* shows an exceptional high concentration of chlorophyll, carotenoids, protein, carbohydrate, antioxidant properties and anticancer activity. It have a major role in bioremediation, *Euglena* accumulate aluminium, zinc, manganese, copper (Pradhan, 2008). It represents an important nutritive base and possess significant effect on the biological productivity in a water body (Padmavathi, 2007).

An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen (Swaran, 2009). The function of pigments as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans (Van den Berg et al., 2000). Therefore, algae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected that they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature (Dufossé et al., 2005). Carotenoids and fatty acids are two non-enzymatic classes of substances which are able to protect the organism from oxidative damage (Sies and Stahl, 1995). The use of methanol as extract solvent restricts the cellular compounds to scavenge the DPPH radical. *E. caudata* and *E. viridis* have been documented to show high biomass concentration throughout the year in freshwater ecosystem (Kim and Boo, 1996).

Plants employ antioxidants for defence against reactive oxygen species (ROS; oxidants) generated during photosynthesis. Plant-based dietary antioxidants are hypothesized to have an important role in maintaining human health. There have been many reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic and antimitotic activities (Salvador, 2007). Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids, fucoxanthin, astaxanthin, mycosporine-like amino acids (mycosporine-glycine) and catechins,
and tocopherols (\(\alpha\), \(\beta\), \(\gamma\), \(\delta\)-tocopherols) (Yuan, 2005). A marine algae, *Sargassum siliculosum* has been demonstrated to prevent the initiation of free radicals that cause cellular damage *in vitro* (Corpuz, 2013). Marine algae are indeed among the richest natural sources of known and novel bioactive compounds that have shown pharmacological activities for many of the deadly diseases. There are very few reports on the antioxidant capacity of algae. Algae (marine and fresh water) that have been used as sources of antioxidants include *Dunaliella salina, Haematococcus pluvialis, Spirulina and Chlorella*. Several species of microalgae, especially green algae, accumulate high concentrations of carotenoids such as \(\beta\)-carotene, astaxanthin and canthaxanthin. *Euglena gracilis* produces significant amount of \(\alpha\)-tocopherol. These carotenoids holds potential for wide application as natural colourants and antioxidants (De and Devasagayam, 2005; Cervantes-García, 2011). The search for natural antioxidant compounds has gained considerable momentum in the recent past that spurred large number of publications on antioxidants and oxidative stress. Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and ageing processes (Kohen and Nyska 2002), which explains their considerable commercial potential in medicine, food production and the cosmetic industry. Further concern about the potential toxic effects of synthetic antioxidants is driving consumer preference for natural products. While antioxidant benefits associated with various terrestrial plants have long been established, the merits of consuming marine macroalgae in this context has been inadequately addressed. The numerous potential human health advantages associated with the utilization of marine macroalgae containing an assortment of antioxidant compounds depends upon both the respective intake of the plants, and the bioavailability of anticipated antioxidant activities (Manach et al., 2004), corresponding aspects of freshwater algae has not addressed properly.

Quite apart from antioxidant activity of algae, such microorganism has recently drawn recurring interests of researchers cutting across various disciplines in the context bionanomaterials. Biosynthesis of metallic nanoparticle using algae and utilization of natural carbonaceous material to access carbon nanomaterial provide a heuristic approach toward novel application of such materials in electronics, drug delivery, catalysis, energy production and storage and also as biosensors, diagnostic tools, drugs and therapies. Devising preparative strategies for carbon nanomaterials from natural plant based resources rather than usual chemical or physical routes are fast becoming popular option. Algae, being whole cell organism with hardly any extraneous
parts like that of higher plants furnish a precursor of almost uniform composition ideal for accessing carbon nanomaterial through Chemical Vapor Deposition (CVD) method (Tessonnier, 2011).

The North-East region of India has been described as a biodiversity hotspot harbouring different kinds of flora and fauna unique to this region. Cachar district in Southern Assam harbour a rich yet unexplored algal blooms in different aquatic habitats. Identification, isolation and characterization of these important communities using polyphasic approach (morphological, physiological and biochemical method) constitute a rewarding theme of research. Considering immense medicinal potential like anticancer, antioxidant, some high value bio-product like pro-vitamins, pigmentation cream, nutritive based product can be made from this flagellate organism.

Admittedly studies on algal blooms of North-East region did not quite receive due attention. Very little information is available regarding the algal blooms. Most of the researchers concentrated on algal diversity and seasonal distribution. Systematic study of a single organism right from the microscopic observation to biochemical properties from a freshwater ecosystem is scanty. Set in this backdrop, the present Ph.D. research work describes ecology, biochemical characterisation and the prospects of *Euglena* found in freshwater ecosystem of Cachar district, North- East India as a a source of carbon nanomaterial. The present thesis includes distribution pattern, diurnal behavior, biochemical properties of *Euglena* bloom including antioxidant property. Innovative effort to prepare carbon nanomaterials from *Euglena*, as a promising natural resource and assess their (CNM) antioxidant activity has also been explored. The work embodied in the present thesis have been organised systematically over nine Chapters with the following sub-themes.

i. To investigate the ecology including diurnal behavior of algal blooms

ii. To evaluate and characterize the bloom acquiring habitats

iii. To carry out chemical characterization of the algal blooms

iv. To assess the algal bloom as feeds some local fishes

v. To assess the phytochemical total antioxidant activity of the algal species

vi. To synthesise Carbon Nanomaterial from the algal biomass by CVD

vii. To collate the results and draw useful generalization