# General Introduction

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## 1.1 Pulp and paper industry

The invention of paper and development of paper industry with its extensive uses played a pivotal role in the development of human civilization worldwide. Paper is more than an industrial product and the per capita consumption of paper is considered as cultural barometer of a nation (Mathur *et al.*, 2009). Of the various aspects of paper, including its crucial role for pedagogic and packaging value, the paper industry has accorded as “core sector” among the process industries as per the Industries (Development & Regulation) Act, 195 1(Rao, 2012). The pulp and paper industry produce over 304 million tons of paper per year. Global production in the pulp and paper industry is expected to increase by 77 % by 2020 and over 66 % of paper will be recycled at the same time (Lacour, 2005).

**1.1. a. Indian scenario**

Today the Indian paper industry ranked among the top 15 global paper industries. In India, it is one of the 35 high priority economic sectors of the Government of India (Mathur *et al.*, 2009). At present, there are 759 small and big paper mills in the country with an installed capacity of 12.7 million tonnes producing 10.11 million tonnes of a variety of different paper, paperboard as well as newsprint products and contributing around Rs. 2500 crores per annum to the national exchequer (Planning Commission 2008). The number of paper mills has increased from just 17 units in 1951 to 715 units in 2009-2010. During this period, the growth in production has increased from less than 0.15 million tonnes to 8.6 million tonnes (Selvarthi and Ramasubramanian, 2010). The pulp and paper industry converts fibrous raw materials into pulp, paper and paperboard using conventional mechanical or chemical methods (Sridach, 2010). Formation of paper from these fibers, considered as an art upto the end of the nineteenth century, has now been fully mechanized. However, as compared to an average consumption of 28 kgs and 58 kgs in Asia and world respectively, the per capita consumption of paper in India is very low i.e. 8.3 kgs as of 2008. The per capita consumption in China is 42 kgs and a staggering 350 kgs in developed countries. The per capita consumption is expected to increase to 12 kgs by 2020 (Xavier and Stella, 2010). In terms of the regional distribution of pulp and paper industry in different states of the country, two large mills are located in Assam.

**1.1. b Environmental impact of pulp and paper mill**

Although has long been accepted as a hallmark of civilization, industrialization is exclusively associated with various negative externalities related to the environmental pollution (Sarma, 2014). The pollution occurs both at the level of industrial production as well as end use of the products and run-off (INSA, 2011). Amongst the various industries, pulp and paper mills are categorized as one of the 12 most polluting industries in India (Verma *et al.*, 2005). Historically, pulp and paper industry has been considered to be a highly intensive sector (in terms of consumption of raw material, chemicals, energy and water) and exerts considerable negative impacts on air, water and soil and as well as on human health (Sarma, 2014). The treatment of toxic wastes, in an economically and environmentally acceptable manner has become one of the most challenging issue being faced by the modern paper industries. These mills deals with a large amount of processed water under mill operation conditions and generation of various toxic and non-biodegradable organic materials such as sulphur compounds, pulping chemicals, organic acids, chlorinated lignins, resin acids, phenolic, unsaturated fatty acids and terpenes etc. (Zhang and Chuang, 1998). The sector is very water-intensive and ranks third in terms of fresh water withdrawal (Devi *et al*., 2003) polluting the surrounding environment. Worldwide, water bodies are the primary dump sites for disposing the pollutants especially from the industries near them. It is estimated that about 300 $m^3$ of wastewater

(Subrahmanyam, 1990) is generated per ton production of paper containing approximately 700 organic and inorganic compounds (Karrasch *et al.*, 2006). The untreated or allegedly treated wastewater poses great environmental concern, as the indiscriminate release of these hazardous wastes into the receiving water bodies lead to numerous environmental disturbances with potential stress to biotic community (Ahluwalia *et al*., 1989). The consequences are manifested by intensification of the levels of these pollutants with time and subsequent shift of indigenous micro floral population like bacteria, algae etc. towards more resistant species such as the cyanobacteria that dominate the wastewater (El-Bestawy, 2008). Phytoplankton species are found to exert different degrees of sensitivity to various pollution stresses (Kott and Wachs, 1964). The type of algae present and their abundance in an aquatic system can reflect the trophic status of that particular habitat and may be indicative of contamination from the addition of nutrients from agriculture run-off or industrial sewage. Recently, biological methods for wastewater treatment have drawn popularity over the traditional physico-chemical processes as well as their outstanding capacity to decontaminate a wide variety of contaminants normally present in the wastewater (Mallick, 2002). Several reports deal with the abundance of cyanobacteria in paper mill wastes (Kirkwood *et al.*, 2001, Vijaykumar *et al.*, 2007, Rout *et al.*, 2010, Saikia *et al.*, 2011, Paranthaman and Karthikeyan, 2013). However, the beneficial application of cyanobacteria for decontamination of industrial effluents is yet to be optimally exploited. In Barak Valley, the *paper mill at Panchgram is* one of the two paper mills of Hindustan Paper Corporation under public sector unit in Assam with annual production capacity of 1,00,000 tonnes. Effluents loaded with high concentrations of toxicants to the nearby river Barak. Lime sludge wastes are dumped outside the mill area. Thus it was felt worthwhile to undertake the present study with an objective to assess the remediation capacity of some native cyanobacterial species isolated from paper mill wastewater toward different contaminants polluting the river Barak.

### 1.2 Diversity and importance of algae

Algae, the pioneer oxygenic prototroph on earth are key contributors to the global photosynthetic productivity (Arunakumara and Zhang, 2009). As a primary producer, algae are accounting for well over 50% of the global carbon budget and forming the basis of the food chain for more than 70% of the world's biomass (Brodie *et al*., 2007; Day *et al*., 1999). They are considered as most diverse and ubiquitous organisms as judged by their widespread occurrence, frequency, abundance and morphological diversity. They are important in the various functional processes and in the cycling of nutrient elements in the ecosystems (Whitton, 1992).

#### 1.2. a. Distribution and diversity of Cyanobacteria

Among the eight major groups of algae, Cyanophytes or blue-green algae are the phototrophic organisms of the superkingdom prokaryotes while other algal groups are eukaryotic. Considered as amongst the earliest phototrophic prokaryotes, the existence of cyanobacteria can be traced back to some 3.8 billion years, performed an important role in the evolution of global biodiversity (Kulasoorya, 2011). They were first named as blue-green algae by Sachs in 1874 because of their water-soluble phycocyanin, phycoerythrin and β-carotene, the precursor of vitamin A pigments (Koksoyi and Aslim, 2013). There are nearly 2000 species of cyanobacteria (Koksoyi and Aslim, 2013) belonging to the class Cyanophyceae under the orders *Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales* (*Oscillatoriaceae, Nostocaceae* and *Rivulariaceae*) consisting over 150 genuses (Rippka *et al*., 1979). According to Rippka *et al*. (1979), cyanobacteria may exist in several forms that may either be unicellular (single or forming colonial aggregates) or filamentous (possessing or lacking heterocysts and akinetes). Unlike other bacteria, they are capable of photosynthesis by using light energy from the sun however, similar to bacteria, cyanobacteria lack a nucleus, and their DNA is spread across the cytoplasm instead of being confined to a single location within the cell (Koksoyi and Aslim, 2013). The adaptive mechanism in cyanobacteria is attributed to osmoprotective compounds and maintenance of low internal contents of inorganic ions which protects the macromolecules of the cell against the environmental stress (Nagasathya and Thajuddin, 2008). Also the presence of cell envelope with three layers as well as an outer sheath enables the organism in buffering the cells from the exposure of direct environmental conditions.

Cyanobacteria are remarkably well adapted to a wide range of environmental conditions occupying almost all ecological niches (Wilkie *et al*., 2011). Both geographical location and local environmental factors contribute to variation in growth pattern of algae and thus they show wide variation in terms of their distribution, ecology, periodicity and as well as in their qualitative and quantitative occurrence (Vijaykumar *et al*., 2007). Apart from the available nutrients, acting as the key controlling factor of cyanobacterial abundance and composition, other physico-chemical factors as light, temperature, salinity, pH, hardness etc. are also of particular importance (Mahar *et al*., 2000). Owing to high genome plasticity they can withstand extreme environmental stressed conditions fairly well

(Tiwari *et al.*, 2005). Due to their ability to endure extreme environmental conditions, algae are often termed as “extremophiles" (Drobac *et al*., 2007). Cyanobacteria are widespread in both terrestrial as well as aquatic localities with diverse trophic status with enormous range of ecological conditions. The ability of cyanobacteria to fix $N_2$ either independently or in symbiosis with other organisms have immense significance in sustenance of fertility in the cropping system particularly rice (Kulasoorya, 2011). Algae represent the pioneer community to colonise bare soil and the nature of algal flora in different localities is the result of a complex influence of soil properties and microclimatic conditions (Metting, 1981; Lukesˇova´, 1993). They are quite common in the aquatic and terrestrial ecosystems though extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (Fogg *et al*., 1973), polluted water bodies, garbage dumps etc. also harbours these organisms (Kulasoorya, 2011). Occurrence of algae in industrial wastewater have been rather extensively studied (Kirkwood *et al*., 2001; Boominathan, 2005; Jeganathan, 2006; Vijayakumar *et al*., 2007; Rout *et al.*, 2010; Dubey, 2011; Vijayakumar *et al*., 2012). Researchers have reported dominance of various cyanobacterial strains acclimatized at higher concentrations of different industrial effluent. These are the genus *Oscillatoria* and *Phormidium* (Singh *et al*., 1969), *Oscillatoria* (Rai and Kumar, 1976), *Oscillatoria, Phormidium, Geitlerinema*, and *Pseudanabaena* (Kirkwood, 2001), *Oscillatoria, Phormidium* and *Lyngbya* (Vijayakumar *et al*., 2007), *Oscillatoria* (Rout and Sarma, 2010) and *Synechococcus, Cyanothece, Oscillatoria, Nostoc* and *Nodularia* (Dubey *et al*., 2011). Cyanobacterial treatment have been successfully employed worldwide as a low-cost and eco-friendly method for remediating different kinds of environmental pollutants from contaminated sites (El-Bestawy, 2008).

### 1.3 Role of cyanobacteria in bioremediation

Bioremediation is a pollution control technology that uses biological systems to catalyse the degradation, reduction or transformation of various toxic chemicals present in soils, sediments, water, and air to less harmful forms. In biological treatment method, relatively little amount of product is produced by resolving a large amount of toxic elements contained in wastewater into carbon dioxide to be stabilized, or by removing organic matters with the generation of methane gas. Through the biological process, pollutants in wastewater can be resolved, detoxified and separated by using microorganisms (Kaushik, 2015) and the contaminants are used up by the microorganisms as nutrient or energy sources (Hess *et al.*, 1997; Agarwal, 1998; Tang *et al.*, 2007). Bioremediation of the effluent is not only species specific, but also dependent on their local environment, nature and concentration of pollutants, exposure time and application as individual or mixed cultures (Dubey, 2011)). Bioremediation has been in use since centuries with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891 (NABIR Primer, 2003). This technology relies on promoting the growth of indigenous microbial consortia of the contaminated sites which are able to perform desired activities (Agarwal, 1998). Amongst the microorganisms, cyanobacteria are now a day's getting more preferences as pollution control agents over the other biological agents, as they are found to be more promising due to their very fast growth rate, simple nutrient requirements, natural resistance and high multi-metal binding capacity (Vijayakumar, 2012). Cyanobacteria are globally recognized as excellent model systems with novel genes and biomolecules having diverse uses in agriculture, industry and environmental sustainability (Prasanna *et al.*, 2008). Thus, cyanobacteria play a duel role of decontamining the waste water and also serving as a potential source of food, feed, fertilizer, and fuel is the key requirement (Subramanian and Uma, 1996). As emphasized by Palmer (1959), to be the reliable indicators of pollution, algae have been used extensively worldwide as a biological monitoring agent to monitor the ecological status of different polluted environments for their outstanding capacity of tolerance for different degrees of pollution. The nature of the effluents can be assayed by algae since the response can be measured in terms of biomass production or through metabolic response generated (Arunakumara and Zhang, 2009). Cyanobacteria play a crucial role as pioneer colonizers on bare rock and soil and modifiers of the degraded ecosystems including various industrial solid waste dumps (Schwabe *et al.*, 1971) through reclamanation of the waste substratum by binding large amount of metals and thus help in vegetational succession (Metting *et al.*, 1981). Lime sludge waste is one of the largest solid by-products generated by the kraft paper mill recovery unit and constitutes a major source of soil pollution (Battaglia *et al.*, 2003; Geng *et al.*, 2006). The lime sludge waste dump is characterized by a unique substrate quality, like very strong alkaline pH (~12). There are some reports available on the occurrence of cyanobacteria in highly alkaline pH (El-Gamal, 1995; Kroll, 1990; Singh *et al.*, 1995; Manchanda and Kaushik, 2000; Gimmler and Degenhardt, 2001). Role of cyanobacteria in reclamation of highly sodic and alkaline soil has been well documented by Kaushik and his groups (Subhashini and Kaushik, 1981; Kaushik, 1985; Kaushik, 1989; Kaushik, 1994). The bioactive compounds produced by algae enhance the physical and chemical properties of soil and thus affect the other components of soil biota (Schwabe *et al.*, 1971). The application of cyanobacteria have been shown to have immense potential in decontamination of different effluents – industrial, domestic effluents, wastewater, terrestrial habitats and heavy metals (Caims and Dickson, 1971). Heavy metals in all the environmental matrices persist due to their potential for persistence, accumulation and biomagnifications in the food chain (Kong, 1995). In high concentrations, heavy metal ions react to form lethal compounds in cells (Nies, 1999). Dias *et al*. (2002) defined metals with atomic masses between 54.63 and 200.59 and special weights of more than 5 g/cm3 are classified as heavy metals. These elements are natural components of earths' crust, which are released into environment because of the natural and as well as human activities. In biology, heavy metals are the atoms with toxic effects such as: Al, As, Br, Cr, Co, Cu, Cd, Fe, Hg, Ni, Mn, Pb, Se and Zn (Imani *et al*., 2011). Some of the metals like Cu, Fe, Mn, Ni and Zn are essential as micronutrients for the life processes in animals and plants while many other metals such as Cd, Cr, Pb and Co have no known physiological activities (Kar *et al*., 2008; Suthar and Singh, 2008; Aktar *et al*., 2010). The main natural sources of metals in waters are chemical weathering of minerals and soil leaching. The anthropogenic sources are associated mainly with industrial and domestic effluents, urban storm, water runoff, landfill, mining of coal and ore and inputs from rural areas (Kabata–Pendias and Pendias, 1992; Biney *et al*. 1994; Zarazua *et al*. 2006). The presence of different algal groups in different proportions in contaminated areas provides a precise idea about the health of that particular ecosystem and thus has been used as means to detect the anthropogenic impact in an aquatic system (Ector and Rimet, 2005). Presently biomonitoring and indeces have become an integral part of water quality assessment and pollution studies (Mahadev *et al*., 2005; Kannel *et al*., 2007). Manahan (1997) in his Environmental Science and Technology reported that certain algal forms grow in the special type of polluted water and these species are characteristic features for the particular environment. Daniel *et al.* (1979) reported the genus *Oscillatoria* to be the most tolerant form of cyanobacteria when exposed to different metal concentrations. The main role in the metal chelation is carried out by the negatively charged groups of the acidic polysaccharides present in the cell envelope, such as carboxyl (Hamdy, 2000; Inthorn, 2001) or sulphate groups. Thus, the potential of the charged macromolecules present on the external layers of cell envelopes (i.e., lipopolysaccharide, capsule, slime) to attach heavy metals seems to be quite promising, giving the possibility of the cyanobacteria to use them either as microbial cultures or dried biomass for the removal of the toxic elements. Yee *et al*. (2004) described the basic mechanism of metal uptake in cyanobacterium involves an initial rapid phase in which ions bind to cell wall followed by a slower and metabolically dependent uptake in cytosol. According to Turner and Robinson (1995) the metal binding nature of microorganisms is associated with the synthesizing of intracellular low molecular weight metal binding proteins or polypeptide (6000-8000 amu) called metallothioneins (MTs). These “MTs” are rich in cys residue and bind to metal ion in metal thiolate cluster. Greene *et al*. (1987) demonstrated the uptake of metal ions (Cu, Pb, Zn, Ni, Cd and Cr) in *Spirulina platensis* was accompanied by liberation of protons and Ahuja *et al*. (1999) reported the release of magnesium ions during the binding of copper and zinc in *Oscillatoria anguistissima* suggesting an ion exchange mechanism. Since the effluents are rich in nutrients due to the loading of organic wastes, they provide ideal habitats for cyanobacteria. Therefore, the study on the floristic pattern and ecology of waste water algae and other microbes is of key importance to understand the fundamental problem created on account of pollution. Cyanobacteria have been shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants, including pesticides (Megharaj *et al*., 1994), crude oil (Sokhoh *et al*., 1992; Al-Hasan *et al*., 1998), naphthalene (Cerniglia *et al*., 1980a, b), phenanthrene (Narro *et al*., 1992), phenol and catechol (Ellis, 1977; Shashirekha *et al*., 1997) and xenobiotics (Megharaj *et al*., 1987). Worldwide, cyanobacteria have now been used efficiently as a low-cost method for remediating dairy wastewater by converting the dissolved nutrients into biomass (Lincoln *et al*., 1996) and for biotreatment (removal) of dissolved inorganic nutrients from fish farms (Duma *et al*., 1998), to allow them to be used as economic and low-maintenance remediation technology for contaminated systems. Several species of microalgae particularly cyanobacteria such as *Oscillatoria, Phormidium, Aphanocapsa* and *Westiellopsis* have been successfully used for the treatment of effluents from various industries. These studies concluded that cyanobacteria efficiently take-up nitrogenous compounds and phosphorus from the effluents and thereby reducing the pollution load (Vijayakumar, 2012). To develop proper and proficient treatment systems, it is mandatory to understand the mutual influence and interactions between the effluents and organisms, so that manipulations to improve the treatment systems become possible. Therefore, in the present study, cyanobacterial cells, capable of growing in waste water have been chosen and their role on the physicochemical properties of the effluent was studied. In turn, the influence of the effluent on the biochemistry of the cyanobacteria to an extent possible was also investigated.

### 1.4 Biochemical properties and application of cyanobacteria

Highly significant ingredients of cyanobacterial biomass are pigments (Desmorieux & Decaen, 2005). Amongst the different groups of algae, cyanobacteria contain the three broad categories of pigments namely chlorophyll *a*, caretonoids and phycobilins for capturing different wavelengths of light (Reis *et al.,* 1998). Cyanobacteria play a significant role in the production and composition of the photosynthetic pigments. Chlorophyll, a key biochemical component in the molecular apparatus, is a cyclic tetrapyrolle, which is similar in structure to that of hemoglobin with the exception that the central metal is magnesium versus iron (Jortner and Pullman, 1999). Chlorophyll *a* is a pigment that is found in most photosynthetic organisms, so its quantification is used as a standard means of estimating phytoplankton biomass and productivity.

Carotenoids constitute a class of natural isoprenoid pigments is required for photosynthesis, photoprotection and the production of carotenoid-derived phytohormones (Cazzonelli, 2011). More than 750 structurally defined carotenoids are reported from nature. Carotenoids provide distinctive yellow, orange and some reddish colours as well as several aromas in plants. All oxygenic photosynthetic organisms including cyanobacteria generally synthesize b-carotene and or its derivatives such as zeaxanthin, violaxanthin, neoxanthin, fucoxanthin and peridinin as major components while some species lack part of these and some contain additional carotenoids, such as canthaxanthin and oscillol dipentoside (Takaichi, 2011). Biological properties of carotenoids allow for a wide range of commercial applications and the antioxidant properties exhibited by these class of compounds constitute at present its core interest (Guedes, 2011). Carotenoids exhibit a variety of pharmacological properties and their uses as a dietary nutritional supplement and as well as precursors of vitamin A are growing rapidly. In human, derivatives of dietary carotenoid promote health by improving different physiological metabolisms and are essential for reproduction (Johnson 2002). In addition, carotenoids have traditionally been used as food additives, animal feed as well as in textiles for their color properties (Cazzonelli, 2011). Now a day's five major types of carotenoids are being manufactured commercially with astaxanthin is getting prior importance due to its multifarious implications as natural colorant (in fish like salmon), therapeutic agent treating cardiovascular disease and prostatic cancer (Fassett and Coombes 2011).

Generally attached on the stromal surface of the thylakoid membranes in prokaryotic cyanobacteria, phycobilliprotein represents the most abundant soluble proteins and the major light-harvesting antennae for photosynthesis. Phycobilliprotein contains linear tetrapyrrole rings attached to apoprotein through thioether linkage (Middepogu, 2012). The phycobilliprotein is a hydrophilic, brilliantly colored and stable fluorescent pigment protein that can be classified into three main groups: Phycocyanin (deep blue), phycoerythrin (deep red) and allophycocyanin (bluish green) depending on the inherent color and absorbance properties (Raja *et al*., 2008). They are characterized by a remarkable degree of spectral adaptations covering the range from 480 to 670 nm and by a combination of energetic and spatial ordering, and low internal conversion rates of the chromophores. The phycobi1isomes are an antenna system working with quantum efficiencies approaching 100% (Jortner and Pullman, 1999). The phycobilliproteins are made up of dissimilar α and β polypeptide subunits (Raja *et al*., 2008). Recent studies have demonstrated the role of phycobilliproteins in hepatoprotective (Ferreira *et al*., 2010), anti-inflammatory (Ferreira *et al*., 2010; Deng and Chow, 2010) and antioxidant

(Ferreira *et al*., 2010; Gantar *et al*., 2012) as well as being a free radical scavenger (Gantar *et al*., 2012).

Cyanobacteria are recognized to be prolific producers of bioactive compounds with anticancer, antibacterial, antifungal, antiplasmodial, algicidal and immunosuppressive properties (Bhadury *et al*., 2004; Dahms, 2006; Abed, 2009 and Rastogi, 2009) drawing interests as a source of various nutraceuticals, biomass and pigments (Pulz and Gross., 2004). Protein rich cyanobacteria like *Anabaena*, *Nostoc* and *Spirulina* are widely used as health food for human beings and also as animal nutritional supplements (Spolaore *et al*., 2006). The cyanobacterium *Phormidium valderianum* has been recently shown to be extremely rich in phycocyanin content (Singh *et al*., 2012). Annually more than 3000 tons of dry weight of *Spirulina platensis* is produced worldwide for extracting phycobiliproteins (Spolaore *et al*., 2006). The ability of some cyanobacteria to accumulate intracellular polyhydroxyalkanoates (PHA) has been found to be used in the production of biodegradable plastics (Steinbüchel *et al*., 1997). The presence of natural photoprotectants such as mycosporine like amino acids (MAAs) and scytonemin protects the cyanobacteria from the harmful ultraviolet radiation (UVR) (Fleming *et al*., 2007; Sinha *et al*., 2008; Singh *et al*., 2008 and Rastogi *et al*., 2010). Commercially cultivated microalgae are acting as an important source of nutritional supplements such as *Chlorella* rich in vitamin C, *Dunaliella*, high in beta-carotene (Horincar *et al*, 2011). Recently, establishment of several plants under the project Biotechnological and Environmental Applications of Microalgae [BEAM] producing *Haematococcus pluvialis* as a source of astaxanthin have been developed in India (Spolaore, 2006).

The content and composition of biochemical properties in cyanobacteria are influenced by nutrient availability and environmental factors such as light, temperature, water and pH (Prassana *et al*., 2004). Reddy *et al*. (1983) stated that the effluent affects algal metabolism at multiple sites resulting in significant reduction of the different biochemical metabolites. However, Monoharan and Subramanian (1996) reported the physiochemical and biochemical alteration in *Oscillatoria pseudogeminata* var. *unigranulata* while treated with paper mill effluents with two-fold increase in carbohydrate value than the control. Vijayakumar *et al.* (2005, 2007) observed decrease protein level in cyanobacteria when treated with different effluents from paper mill. Reddy *et al.* (1983) observed an increased concentration of oil refinery effluent decreased the biochemical contents such as proteins and amino acid in blue green algae as compared to control. Similarly, Ganapathy *et al.* (2011) observed an increased concentration of distilleries effluent suppressed the carbohydrate, protein, amino acid and lipid of *Nostoc muscorum*. Fathi *et al.* (2005) demonstrated that the elevated metal concentrations severely suppress both the chlorophyll contents and protein level resulting in increased carbohydrates. Similarly, Walach (1987) stated that under constant carbon availability and nitrogen deficiency, carbohydrate synthesis increased. Studies have shown that the wastewater polluted with heavy metals could enhance or stimulate the protein content of the cyanobacteria. The level of protein is found to be enhanced at the lower level of heavy metal while decreased at the higher level (Rai *et al.*, 1994, Hart and Scaife, 1997). El-Sheekh *et al.*, (2011) reported stimulation of protein synthesis when treated with lower concentration of copper which may be attributed to the synthesis of stress proteins against metal toxicity while the depletion of protein at higher metal contents may be attributed to shortage of carbon skeleton results from low photosynthetic rate.

### 1.5 Molecular aspect in cyanobacterial taxonomy

The classical methods for cyanobacterial taxonomic classification involved microscopic examination based mainly on morphology (Gietler, 1932; Rippka *et al.*, 1979; Desikachary, 1059). However, in many cases the classification was known to be incongruent with the phylogeny of cyanobacteria because morphology can vary considerably in response to fluctuations in environmental conditions (Anagnostidis and Komárek, 1989). Also, the perennating bodies of cyanobacteria such as hormogonia, akinetes and heterocysts may be difficult to characterize by microscopic observation and thus the actual diversity can be underrated (Nübel *et al*. 2000). In addition, the phenotype of many cyanobacterial strains alters during prolonged laboratory cultural conditions. In view of the above, cyanobacterial diversity assessments and community analysis investigated by microscopic observation was supplemented with a molecular taxonomy.

The whole classification system of cyanobacteria (species, genera, families, orders) has undergone extensive restructuring and revision in recent years with the advent of phylogenetic analyses based on molecular sequence data. As anticipated by Komárek (2010), several recent revisionary and monographic works initiated a revision and there will be further changes in the future. The phylogenetic clustering of strains of several cyanobacterial genera seem to be incongruent with the cyanobacterial morphology and does not follow their current classification [e.g., *Anabaena* and *Aphanizomenon* (Lyra *et al*. 2001; Gugger *et al*. 2002), *Oscillatoria* (Suda *et al.* 2002) and picocyanobacterial genera such as *Synechococcus* and *Synechocystis* (Wilmotte and Herdman 2001)]. In some cases strains of a genus or species formed a monophyletic cluster in the 16S rRNA gene analysis, for example *Microcystis* (Otsuka *et al*. 1998; Lyra *et al*. 2001), *Planktothrix agardhii* (Lyra *et al*. 2001) and *Nodularia* (Lyra *et al*. 2005). However, the morphologically distinguished *Microcystis* species were found to be genetically very closely related to each other (Otsuka *et al.* 1998). Unification of different *Microcystis* species into a single species has been proposed (Otsuka *et al*. 1998, 2001). Thus, a combination of attributes, including morphological (trichome form/shape, vegetative cell width, position/shape/size of heterocysts and shape of terminal cell) and biochemical/ physiological (PBS, nitrogenase, GS and ammonia excreting activity), besides molecular profiling, would provide useful taxonomic criteria for distinguishing among the isolates belonging to the same genus when analyzed under a uniform set of conditions. Such attributes if included in the descriptions of the genus/species/strains in taxonomic treatises, can provide a more comprehensive characterization of the members placed under the particular genus. This would facilitate not only selection of particular strain(s) for detailed analyses of various metabolic processes but also for their efficient utilization for miscellaneous purposes (Prasanna *et al*., 2006). The first reconstruction of the phylogenetic interrelationships among cyanobacteria (Giovannoni *et al*., 1988) was based on 16S rRNA of organisms maintained in axenic cultures, provided an insight in early diversification of the group. This system has been compared with morphotypic expression of the examined taxa and found some significant correlations, but identified also the polyphyletic nature of both traditional botanically as well as bacteriologically established genera (Wilmotte, 1994). Since then, the GenBank has been augmented with numerous complete and partial sequences of the 16S rRNA gene derived from both axenic and non-axenic uni-cyanobacterial cultures, as well as cyanobacteria from natural populations. This data provides a functional matrix within which the overall phylogenetic relations were reconstructed (Rudi *et al*., 1997; Wilmotte & Herdman, 2001).

### 1.6 Significance of the present study

Monitoring and controlling river water quality have become key issues in recent years. The river Barak is the second largest river after Brahmaputra in Assam and Northeast India. This riverine system is the life line for the people of Barak valley lying in the southern part of the state of Assam and providing water for domestic, industrial and agricultural irrigation practices in addition to fishery and recreational activities. However, the river is subjected to high levels of pollution due to different degrees of anthropogenic activities such as indiscriminate disposal of domestic and agricultural wastes and also release of large quantities of liquid wastes from Cachar paper mill sited on its bank near Panchgram is of particular concern over the years. The release of high organic and inorganic loads discharged from both point and non-point sources has resulted into the alteration of the water quality in terms of objectionable colour, odor and high biochemical oxygen demand, nitrate, phosphate and lowered dissolved oxygen contents of the river hence make the water unsuitable for domestic use and posing thread to the aquatic life. Therefore, with the limited availability and increasing pollution of the water, it has become imperative to make the water reusable by removing the pollutants and therefore wastewater treatment has become both an ecological as well as economical necessity. Biological treatment of wastewater is a growing field of active research mainly by using microalgae including cyanobacteria. Cyanobacteria are the major microbial indicator of water quality monitoring and are widely used for assessment of water quality and purification of polluted water. Isolation and utilization of the indigenous cyanobacterial biomass for remediation processes of highly toxic pollutants offers a very efficient and cheap tool for governmental or private industrial activities. Besides very few sporadic accounts of algae and water quality assessment of the river Barak, application of local effluent grown microbial flora for the remediation of waste water being discharged in to this river is really scanty. In this backdrop and in the context of present research it was felt worthwhile to undergo a systematic survey of the algal flora followed by establishment of pure culture and molecular characterization of cyanobacteria and finally their application for waste remedial purpose. Hence the advantageous potential of using the local waste grown cyanobacterial species for the treatment of contaminated wastewater may results in the quality improvement of the discharged wastewater which in turn will eliminate or at least minimize the expected deterioration of the receiving environment. Accordingly, the present Ph.D. research work portrays an account of investigation on distribution of algal communities around Panchgram paper mill area and screening of novel species for bioremeadiation. The chapters are organized systematically with the following objectives.

**OBJECTIVES**

1. To collect and identify algal samples and habitat characterization from strategically identified study sites around paper mill area of Panchgram in Hailakandi district with special emphasis on cyanobacteria.

2. To assess the diversity and ecobiology of cyanobacteria including other groups of algae.

3. To study the growth kinetics, photosynthetic pigment analysis and biochemical characterization of some selected cyanobacterial strains.
4. To perform bioremediation work at laboratory scale to observe the efficiency of the selected species to remove pollutants from the environment.
5. PCR based molecular characterization of selected cyanobacterial strains to understand their phylogeny and development of cyanobacterial germplasm for further studies.