DISTRIBUTION OF ALGAL COMMUNITIES IN AND AROUND PANCHGRAM PAPER MILL, SOUTHERN ASSAM AND SCREENING OF NOVEL SPECIES FOR BIOREMEDIATION

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General discussion

Photosynthetic algae evolved on earth for billions of years ago, are the primary producer of majority of life on the planet. They are considered as most diverse and ubiquitous organisms as judged by their widespread occurrence, frequency, abundance and morphological diversity. Cyanobacteria are remarkably well adapted to a wide range of environmental conditions occupying almost all ecological niches (Wilkie *et al.*, 2011). Owing to high genome plasticity algae can withstand extreme environmental stressed

conditions fairly well. They are common not only in the aquatic and terrestrial ecosystems but also found in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (Fogg *et al.*, 1973) as well as frequent inhabitants of polluted water bodies, garbage dumps etc. which are generally uncongenial to most other organisms (Kulasoorya, 2011). Different researchers have reported dominance of various cyanobacterial strains acclimatized on higher concentrations of different industrial effluent belonging to the genus *Oscillatoria*, *Phormidium*, *Lyngbya, Synechococcus, Cyanothece, Nostoc* and *Nodularia* etc. The application of cyanobacteria showed immense potential in decontamination of different industrial effluents, domestic effluents, wastewater, terrestrial habitats and heavy metals (Caims and Dickson, 1971). Cyanobacteria have been shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants. Now a day's cyanobacteria have been used efficiently as a low-cost method for remediating industrial wastewater by converting the dissolved nutrients into biomass (Lincoln *et al*., 1996) and for biotreatment (removal) of dissolved inorganic nutrients from polluted water. Several species of microalgae particularly cyanobacteria such as *Oscillatoria, Phormidium,* Lyngbya, *Aphanocapsa* and *Westiellopsis* have been successfully used for the treatment of effluents from various industries. **Chapter 4** deals with the seasonal variation and ecological assessment of algal community in selected study sites (river site soil, solid wastes, tree barks, around papermill) of Panchgram area in Hailakandi district. A total of nine physico-chemical parameters of water (water temperature, pH, electrical conductivity, dissolved oxygen, total alkalinity, dissolved silica, soluble reactive phosphorus and nitrate), two microclimatic conditions (air temperature and humidity) and seven physico-chemical

properties of soil samples (pH, conductivity, bulk density, water holding capacity, soil organic carbon, soil organic matter and texture) were studied to analyze the ecological factors affecting the algal growth and diversity including cyanobacteria in the selected study sites of the study area. Seasonal study of the physico- chemical properties of water revealed significant seasonal fluctuations. However site wise seasonal fluctuation was not much significant. pH, free CO_2, nitrate, phosphate, silica were found to be highest in site 2 *ie.* effluent reaching point while transparency and dissolved oxygen was found to be lowest in the same point compared to the other three points *ie.* upstream zone and two mixing zones. Irrespective of the ecosystems, soils were found to be acidic in nature except solid waste deposits (lime sludge). Lowest pH was noticed in river bank soil of Station 2 and highest was obtained for solid waste deposited area *ie.* Station 4 (lime sludge). The lime sludge waste dump is characterized by a unique substrate quality, like very strong alkaline pH (~12). Silt proportion was found to be higher in all the sites except lime soil and soil surrounding tree barks area where sand was higher. A total of 123 algal species under 47 genera were identified in the selected ecosystems of the study area. The highest algal species was obtained in the class Cyanophyceae. Highest number of blue green algal species belonged to the genus *Oscillatoria* (12) followed by *Lyngbya* (6) and *Anabaena* (4). Genera under *Nostocaceae, Scytonemataceae, Rivulariaceae,* and *Oscillatoriaceae* are found to be distributed in all the study sites. The Blue green algae were more abundantly present during the pre monsoon and winter season at all the sites of river bank soil irrespective of all the fields while in case of solid wastes, tree barks and upland soil, abundance of algae was higher during monsoon and premonsoon period. In the soil contaminated by wastewater, non-heterocystous forms were found to be dominant

over the heterocystous forms. Lower availability of heterocystous cyanobacteria indicates the presence of higher level of combined nitrogen (2012; Negi and Rajput, 2013; Kannan, 2008; Boominathan *et al*., 2007; Paranthaman and Karthikeyan, 2013). The relative abundance of blue green algae was highest at Site 8 (89.48%) during premonsoon and least in site 2 (5.19%) during monsoon. Highest algal diversity was observed at station 2 during post premonsoon season with maximum Shannon-Wiener diversity index (H=2.21), minimum Simpson's dominance index (D=0.14) and maximum Pielou's evenness index (J=0.86). The algal distribution at station 5 during premonsoon was observed to be least diverse with minimum Shannon-Wiener diversity index (H=0.65), maximum Simpson's dominance index (D=0.67) and minimum Pielou's evenness index (J=0.43). Culture dependent estimation of cyanobacterial diversity from the soils of the study sites was assessed by plating technique **(Chapter 5)**. Twenty seven strains under 13 genera were estimated. From the isolates 27, 16 strains from wastewater and 7 strains from solid wastes and 4 from tree bark were made unialgal by repeated streaking on the agar plates. Cyanobacteria isolates included 21 heterocystous filamentous, 5 non-heterocystous filamentous and only 1 unicellular strains. Nostocaceae were the most dominant organisms isolated from the soils of the study sites. Maximum number of strains was belonged to the genus *Nostoc* (6), followed by *Anabaena*, *Calothrix*, *Cylindrospermum*, *Phormidium* and *Tolypothryx*, each of which contained 3 strains. The genus *Lyngbya* was represented by 2 species while the other 5 genera (*Fischerella, Nodularia*, *Oscillatoria*, *Scytonema* and *Westiellopsis*) were represented by only one strain each. Growth kinetics, photosynthetic pigment analysis and biochemical characterization of some of the selected cyanobacterial strains isolated from the soils

around Panchgram papermill (river site soil, solid wastes, tree barks, around papermill) of Hailakandi district was studied in **Chapter 6.** Comparatively short lag period was observed for non-heterocystous filamentous forms. However, most of the heterocystous isolates were having a long lag phase which might be due to the adjusting time required by the organisms to the new environment (Madigan *et al.*, 2000) and also for the repairing of macromolecular damages that occurred during last stationary phase (Dukan *et al.*, 1998). Both chlorophyll *a* content and growth rate was generally found to be lower in the organisms under rivulariaceae. Growth rate was found highest in *Oscillatoria formosa* ($0.46d^{-1}$) isolated from the wastewater with lowest generation time ($51.71h^{-1}$) followed by *Nostoc linkia* with growth rate $0.40d^{-1}$ and generation time $59.52h^{-1}$. *Nodularia spumigena* was found to be the slowest grower (growth rate, $0.09d^{-1}$; generation time, 241 h^{-1}) among the isolates. Highest chlorophyll *a* (9.43mg/l) and were observed in *Lyngbya polysiphoniae*. *Westiellopsis prolifica* was recorded for highest TCC, followed by *Scytonema tolypothrichoides* and *Aphanothece bullosa*. *Anabaena orientalis* was recorded for lowest TCC (0.26mg/l). *Oscillatoria formosa* exhibited significantly higher amount of soluble protein followed by *Nostoc linckia* and *Nostoc carneum*. Carbohydrate content is highest *Nostoc linckia followed by Anabaena orientalis and Fischerella musicola.* Maximum value for Phycocyanin (PC) recorded in *Anabaena spiroides* and lowest in the *Tolypothryx byssoidea*. Bioremediation of paper mill effluent using some selected cyanobacterial strains was studied in **Chapter 7**. The purification of the paper mill wastewater subjected to biological treatment using three selected strains namely *Oscillatoria formosa, Lyngbya polysiphoniae* and *Nostoc carneum* was evaluated and compared. In the present investigation, all the selected species of cyanobacteria

remarkably reduced the organic (BOD, COD), physical (TDS, TSS), chemical (chloride, nitrate, phosphate and ammonia) and heavy metals (copper, chromium, cadmium, nickel, cadmium, manganese) contaminants of the effluent sample with the incubation period of 24 days. Copper, chromium, cadmium, lead, manganese and nickel were found in raw effluent were significantly reduced or almost completely removed after treatment. The experimental results confirmed that the most effective species for heavy metal removal from the papermill effluents are in the following order *L. polysiphoniae* > *O. formosa* > *N. carneum* which may be attributed to the selective uptake of the investigated metals by the tested cyanobacterial species. Cyanobacterial species, tested for heavy metal removal is found that *L.polysiphoniae* is able to remove copper and chromium better than other microbes and an increase in the percent metal ion removal by the algae with increasing copper concentrations in the medium was recorded. The average removal of Cu ions for *Oscillaioria sp.* was 44% while that for *Nostoc sp*. was only 37% after 24 days. The average removal capacity of Cr ions for *Lyngbya* was found to be 74%, for *Oscillaioria* sp. it was 63% while that for *Nostoc* sp. was only 30% after 24 days. The results also revealed that *N. carneum* was the most sensitive alga to the two metal ions even at lower concentrations (3 and 5mg/ L for Cu and 5 and 7 mg/L) while *L. polysiphoniae* and *O. formosa* were more tolerant to high metal concentrations. The bioremoval of heavy metal ions by *L. polysiphoniae* from aqueous solution showed that the highest percentage of metal bioremoval was recorded 73% (Cu) and 88% (Cr). From the present study, for both the metals analysed, the % removal of Cu and Cr with increasing metal concentrations shows interspecific variation. *Lyngbya* showed significantly greater sorptive capacity for Cu and Cr than all other strains tested. Absorption Spectra for chlorophyll indicate that

all the algae showed rapid growth up to 15th day in case of control and treated (0.5 mg/L for *Lyngbya* sp., 0.1 mg/L for *Oscillatoria* and 0.3 mg/L for *Nostoc* sp. for copper and 5 mg/L for *Lyngbya* sp., 1 mg/L for *Oscillatoria* and control for *Nostoc* sp. for chromium respectively). Metal treatment favored the growth of all the three cyanobacteria with increasing chlorophyll concentration upto some days, however, exposure of the cyanobacterium beyond these concentrations led to progressive decrease in the growth. LC_{50} values for *Lyngbya*, *Oscillatoria* and *Nostoc* sp. was found to be 0.89 mg/L, 0.69 mg/L and 0.63 mg/L respectively for copper while it was 7 mg/L, 5.8 mg/L and 3.96 mg/L respectively for *Lyngbya*, *Oscillatoria* and *Nostoc* sp. respectively for chromium. For wastewater, LC_{50} values were 79.98 % for *Lyngbya polysiphoniae*, 69.18 % for *Oscillatoria formosa* and 48.98 % for *Nostoc carneum*. Concerning the biochemical parameters of three species, the results revealed that the treatment with low concentration of $CuSO_4$, K_2CrO_4 and wastewater stimulated the biochemical contents, but inhibited the accumulation of biochemical contents at higher concentrations. The toxic effects of lethal concentration of these metals on growth and biochemical constituents were more pronounced in *Oscillatoria* and *Nostoc* sp., than in *Lyngbya polysiphoniae*. A total of 8 cyanobacterial strains **(Chapter 8)** were screened for the molecular characterization. To illustrate the phylogenetic relationship of the isolated strains, 23 sequences of 16S rRNA gene were aligned and the evolutionary relationships were inferred. In the study, 16S rRNA gene was succesfully utilized for cyanobacterial phylogeny as mentioned by earlier workers (Giovannoni *et al.*, 1988; Seo and Yokota, 2003; Arima *et al.*, 2012; Teneva *et al.*, 2012). Furtado *et al.*,(2009) mentioned congruence between morphological and the phylogenetic tree of the isolated strains based on 16S rDNA

analysis. A biological treatment process consisted of native cyanobacteria was applied to pulp mill effluents in order to increase the quality of wastewater. During the present study, the selected indigenous cyanobacterial species performed high efficiencies as suspended growth application toward the removal of both organic (BOD and COD), physical contaminants (solids; suspended and dissolved), chemical contaminants (nitrate, phosphate and ammonia) as well as heavy metals (Cu, Cd, Cr, Pb, Mn and Ni) from the papermill wastewater. Thus, the results obtained in this experiment proved the biotechnological applicability and feasibility of using the tested microalgae for wastewater treatment where promising removal of the investigated contaminants were achieved. Therefore, this study highlighted a novel approach for the application of biological process for a feasible solution for wastewater through quality improvement which in turn will help in meeting the requirements for the wastewater discharge to the water bodies.