CHAPTER-V

SUMMARY

AND

CONCLUSIONS
Paper-mills are one of the major sources of wastewater generation. Since conventional physicochemical treatment methods are cost prohibitive, attempts are being made particularly in developing countries, to search for low cost biological treatment methods. In order to produce suitable and efficient biological treatment systems, it is necessary to understand the mechanism of interaction between the effluent and the organisms so that improvement in the treatment systems can be achieved through manipulation. Algae often serve as excellent indicators of pollution since they respond typically to many ions and toxicants. Indeed, cyanobacteria are ideally suited to play a dual role of cleaning wastewater in the process of effective utilisation of its different constituents essential for growth leading ultimately to enhanced biomass production. The present work describes experimental findings on the interaction of *Anabaena doliolum* Bharadwaj, a fresh water cyanobacterium to the paper-mill effluent. The findings and generalisation of data with the concluding remarks are extensively described in the Result and Discussion sections.

The physicochemical analysis of the paper-mill effluent (Table 2.3) shows the alkaline nature of the effluent containing high BOD and COD. It also contains different nutrients that might be necessary for the algal growth.
Anabaena doliolum was found to grow in 75% effluent supplemented with BG\textsubscript{11}{(o)} nutrients as well as in 100% effluent without nutrients of BG\textsubscript{11}{(o)}, but the growth was relatively slow in 100% effluent without additional nutrients compared to that in 75% effluent with nutrients (Fig.2.1). The 100% effluent without supplementation of BG\textsubscript{11}{(o)} nutrients contains very negligible amount of P which is the major limiting factor for proper growth of the organism. In addition to growth measurements, similar findings were also obtained for pigment (Chl-a and car) (Fig. 3.1 & 3.2) and protein contents (Fig. 2.2). The slow growth rate, low pigment and protein content of Anabaena grown in 100% effluent may be attributed to lack of P and reduction in light intensity due to screening effect by the coloured effluent, high level of COD and Na\textsuperscript{+} ions present in the effluent. 75% effluent supplemented with BG\textsubscript{11}{(o)} derives nutrients like Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Cl\textsuperscript{-} and significant amount of P from the exogenous medium along with N\textsubscript{2} in moderate quantity from the effluent, which are necessary for algal growth. Therefore, 75% effluent treatment works as the stimulatory concentration for the growth of the organism.

The cyanobacterium fixed N\textsubscript{2} appreciably in BG\textsubscript{11}{(o)} nutrient medium but the N\textsubscript{2} fixing capacity of the organism was inhibited when it was grown in media with 100% or even 75% effluent supplemented with BG\textsubscript{11}{(o)} nutrients (Fig. 2.3). The N\textsubscript{2} fixing ability of cyanobacteria is suppressed by supplementary combined N\textsubscript{2} in the culture medium.
Changes in thylakoid organisation in the present study have been assessed by examining the absorption and emission characteristics of the photosynthetic assemblies of the alga grown in different concentrations of paper-mill effluent. A significant reduction in fluorescence emission intensity (F₆₈₅) in 100% effluent treated alga (Table 3.4) indicates the effluent induced possible alteration in the primary photochemistry of PS II. A reduction in the peak heights of the excitation spectra treated with 100% effluent supports the proposition of changes in thylakoid organisation that lead to change in primary photochemical events. The effluent at this concentration results in the low level of Chl in the organism. On the other hand, a relative increase in the peak height of excitation spectra in 75% effluent treated sample is indicative of the normal development of photosynthetic unit which is further supported by the high level of Chl accumulation. An increase in the fluorescence polarisation (p) in 100% effluent treated algal cells compared to control may be due to the formation of rigid lipid structure in thylakoid membranes whereas low level of gel phase formation in 75% effluent treated sample as evidenced from the polarisation data (Table 3.5) indicates no significant change in the status of membrane fluidity.

An enhancement in the PS I transport activity in 75% effluent treated sample indicates the stability and functional efficiency of PS I, whereas a decrease in the activity of the 100% effluent treated alga might
be due to the alteration in the ability of the photosynthetic apparatus for photochemistry (Table 3.6 and 3.7). From the data on the changes in differential efficiency of partial electron transport system it is indicated that whole chain electron transport system with H₂O as electron donor and MV as acceptor remains more efficient than partial electron transport system at reducing side of PS I with DCPIP as electron donor and MV as acceptor. Since the low efficiency of the latter appears to affect the former, the conclusion that could be drawn is that the effluent treatment might be modifying the structure and organisation of plastocyanin/PS I reaction centres that loses free access to exogenous donor and the entire process does not permit it to receive electrons from the reduced DCPIP efficiently.

An increase in the O₂ evolution of algal cells treated with 75% effluent is due to the high efficiency of PS II activity compared to 100% paper-mill effluent treated organism. The 75% effluent supplemented with BG₁₁₀ nutrients contains a significant amount of Mg²⁺, Ca²⁺, Cl⁻ which are the ions necessary to carry out the light reaction of photosynthesis and modulate the structural stability of OEC (PS II) in the thylakoid.

In the 100% paper-mill effluent, due to PO₄²⁻ phosphate limitation, the vegetative cells of the organism are transformed to a special
kind of cells with large cell size, distinct cell wall thickening and central granular structure that are characteristics of akinetes. This transformation is relevant in the light of adaptational response of the organism to the nutrient stress.

One of the recent trends is to search for photosynthetic organisms with high growth rates, high biomass yields and higher utilisation potential which could be mass cultured in wastewater and play a dual role of cleansing the water and also serving as a source of food, fertiliser and fuel. The present study indicates that *Anabaena doliolum* Bharadwaj may be effectively used in the biological treatment of paper-mill wastewater.

CONCLUSIONS

(1) The physico-chemical analyses of paper-mill effluent shows the presence of high BOD, COD, Na\(^+\) and absence of any significant amount of P. However, the effluent is also found to contain different ions that are necessary for cyanobacterial growth, photosynthesis and N\(_2\) fixation. 75% paper-mill effluent contains PO\(_4^{2-}\) in sufficient amount from the BG\(_{11(0)}\) nutrient medium whereas 100% effluent without BG\(_{11(0)}\) nutrient supplementation lacks the PO\(_4^{2-}\) source that becomes a limiting factor.
(2) Present findings, that 75% paper-mill effluent supplemented with the BG_{11(0)} nutrient is the stimulatory concentration enhancing the growth and photosynthetic activities of *Anabaena doliolum* Bharadwaj, indicates that the paper-mill wastewater can be used for algal growth as well as pollution abatement after it is processed.

(3) Processing of effluent with exogenous addition of PO_{4}^{2-} is necessary in the background of negligible amount of it being present in the paper-mill effluent.

(4) The industrial waste primarily contains two important components, one that constitutes of some essential elements that favour the growth of the organism and the other that contains toxic elements. It also lacks a few essential elements like P. The organism can therefore, grow fairly well with the former with its proper adjustment to the latter. For the adjustment, the organism has to develop adaptational strategy. The modifications of cellular structure and organisation induced in the alga by paper-mill effluent in the present work strongly support the proposition.