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Bioremediation of Tannery Effluents by Filamentous Cyanobacteria *Anabaena Flos-Aquae* West

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Abstract

Heavy metal pollution of ground and surface waters by industrial effluents has become a serious threat to the environment especially in developing countries. Though many conventional physicochemical methods are currently being practiced, biotechnological methods are becoming attractive alternatives, as they are economical and eco-friendly. In this study, the search for innovative and eco-friendly biotechnologies to remove toxicants from effluents has focused attention on the detoxification capacity of a variety of microbe's especially cyanobacteria. The treated effluents from tannery industry were collected and added to the cyanobacterial growth medium in various proportions. The photosynthetic pigments and nitrogen status of *Anabaena flos-aquae* were analysed before and after the treatment with effluent. The present investigations showed that *Anabaena flos-aquae* can serve as the potential bioremedial organism for industrial pollution.

Introduction

Industrial wastes are usually generated from different industrial processes. The amount and toxicity of waste released from industrial activities varies with the industrial processes. Again among all the industrial wastes, those released from tanneries have the highest concentration of pollutants [1]. Tannery wastewater treatment is complex due to the variety of chemicals added at different stages of processing of hides and skins. Major problems in tanneries are due to wastewater containing heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salts and other pollutants [2]. Water bodies receiving the tannery effluent show high BOD, COD and chloride levels (Rajan and Rukmani, 2000) that are well above the stipulated concentrations prescribed by the Indian Standard Institute (ISI). Under these circumstances, an alternative and effective way of solving pollution problem has to be evolved using conventional available resources. Some of the plants can resist pollution caused by effluent released from tanneries and in fact utilize the dissolved salts for their growth. Several laboratories all over the world are currently involved in the cleaning of pollutants using microorganisms. They are embarked on as 'bioremediation technology' to establish a clean environment. A few microorganism are also suggested as good 'bioremediators' which have developed a mechanism to withstand the pollutants in the environment and at the same time use them for their sustained growth. 'Bioremediation' is an alternative low cost technology involving the use of plants or microorganisms to remove transform or stabilize the contaminants in soil, water or sediments. Some of the biomasses studied for their efficacy to remove heavy metals and used as efficient biosorbents include, bacteria [3], fungi [4], yeast and marine algae [5]. A few species of marine algae such as *Ascophyllum* and *Sargassum* (Phaeophyceae) are effective in the biosorption of pollutants [6,7]. The major advantage of this technology is that the concentrations of heavy metals in the polluted environment are reduced to a very low level using inexpensive biosorbent materials.

In the present study, photosynthetic nitrogen fixing cyanobacterium, *Anabaena flos-aquae* was used to find out their efficacy in remediating the tannery effluent. It was grown in the normal growth medium amended with various dilutions of tannery effluent and the response of alga in terms of growth and nitrogen status of cells were used as an index of resisting the pollutants in tannery effluent.

Materials and Methods

Collection of Samples

Treated effluents were collected from sampling sites of tannery industries located at Trichirappalli, Tamil Nadu. The samples were collected in sterile borosilicate bottles protected from sunlight during transportation.

Maintenance of Cultures (*Anabaena flos-aquae*)

Axenic cultures of *Anabaena flos-aquae* was kindly provided by Algal Laboratory Culture Collection Centre, established maintained at Centre for Advanced Studies (CAS) in Botany, University of Madras. The culture supplied by Dr. N. Anand, Director, CAS in Botany, University of Madras, was obtained for the present work by Dr. V. T. Sridharan, Former Head of the Department of Botany, National College, Trichy. Axenic cultures of *Anabaena flos-aquae* were grown in Allen and Arnon's medium (Allen and Arnon, 1955) that was free of nitrogen.

Treatment: Log phase cultures of *Anabaena flos-aquae* were used for the treatment. They were inoculated in the N-free Allen and Arnon's medium (Control) with or without the tannery effluent (Treated) to get a final chlorophyll concentration of cultures at 5ug Chl/ml. Cultures were grown in 500 ml conical flasks and kept in

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orbital shaker for uniform growth with an exposure to light intensity of 3000 lux. Tannery effluent collected from the site was sterilized and added to the growth medium in the inoculation chamber suitably to get dilutions of 1:10, 1:100 and 1:1000.

On the 7th day of treatment, cultures were withdrawn, centrifuged and the pellet was analysed for various parameters.

Analysis of Photosynthetic pigments

Photosynthetic pigments were determined in the samples according to the method described by [8] as modified by Mckinney's procedure [9]. The estimation of Cartenoids was performed by Myers and Kratz [10]. Total free amino acids and Total Sugars were determined in the samples using Jayaraman [11]. Total protein was determined by the method of Lowery et al. [12]. The estimation of total nitrogen was performed according to Humphries. Phycobiliprotein (Bennett and Bogorad, 1973), heterocyst frequency [13], photosynthetic oxygen evolution and respiratory oxygen consumption were also evaluated in the samples.

Results and Discussion

The quantitative analysis of chloroplast pigments on the initial and 7th day of treatment revealed that the total chlorophyll & carotenoid (Figure 1) pigments were continued to be synthesized by *Anabaena flos-aquae* under normal growth conditions. When the culture was grown in full effluent, there was hardly any synthesis chlorophyll & carotenoid (Figure 1). Intracellular levels of these pigments indicate that supply

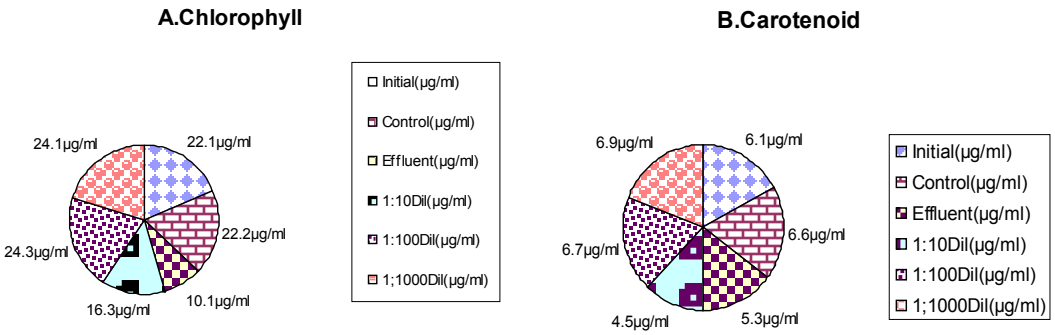

Figure 1: Total chlorophyll (A) Carotenoied (B) content of *Anabaena flos-aquae* cultures exposed to tannery effluent treatments.

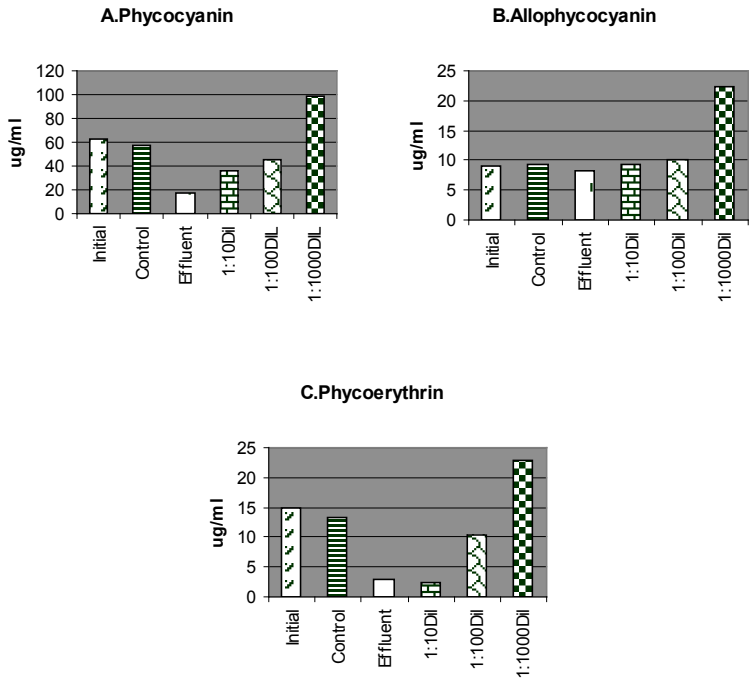

Figure 2: Phycobiliprotein content of *Anabaena flos-aquae* cultures exposed to tannery effluent treatments.

of effluent at 1:10 dilution caused 50% increase in Chl. contents over control (Figure 1A). Dilution of tannery effluent supported the growth as revealed by the enhanced Chl. levels of cultures exposed to 1:100 and 1:1000 dilutions of tannery effluent. Growing the cultures in effluent alone indicated that the level of chlorophyll was brought down to 16.3 ug/ ml from the initial level of 22.1 ug/ml.

Carotenoid content was also analyzed to find out the effects of tannery effluent treatment. As shown in Figure 1B, it is observed that the tannery effluent treatment lowered the intracellular levels of carotenoid from 6.1 ug/ml to 5.3 ug/ml on the 7th day. More amounts of carotenoid could be noted upon dilution to tannery effluent 1000 times. Treatment of cultures with full effluent, 1:10 dilution, and 1:100 dilutions caused the significant reduction in the cellular levels of PC. However, dilution of tannery effluent 1000 times and addition in to the growing medium, enhanced cellular PC content (Figure 2A). A similar effect was observed when APC (Figure 2B) and PE (Figure 2C) were analyzed in cultures of *Anabaena flos-aquae*. In cyanobacteria, phycobiliprotein (PBP) constitutes the bulk of photosystem II reaction centre. PBP is made up of phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). Of these, PC is the dominant pigment in *Anabaena flos-aquae* and it was of interest to find out the effects of tannery effluent on their production.

After 7 days treatment, the dissolved oxygen showed that there was enhancement in photosynthetic rate of cultures treated with the 1:100 and 1:1000 dilutions of tannery effluent when compared to control and full effluent-treated cultures. Respiratory activity was greatly reduced in cultures of *Anabaena flos-aquae* grown in tannery effluent diluted with medium to get 1:1000 dilutions when compared with cultures treated up to 1: 100 dilutions (Figure 3). Heterocyst frequency was measured as % of vegetative cells and the results obtained is indicated in Figure 3C. It was observed that the cultures grown in effluent alone showed low (10%) heterocyst frequency after 7 days of treatment. It remained constant in control cultures. The supply of tannery effluent one after diluting 100 and 1000 times did bring about notable changes in heterocyst frequency of cultures. The heterocyst frequency was brought to 16% and 15% from the initial value of 18%.

Heterocyst is the site of nitrogen fixation in cyanobacteria and

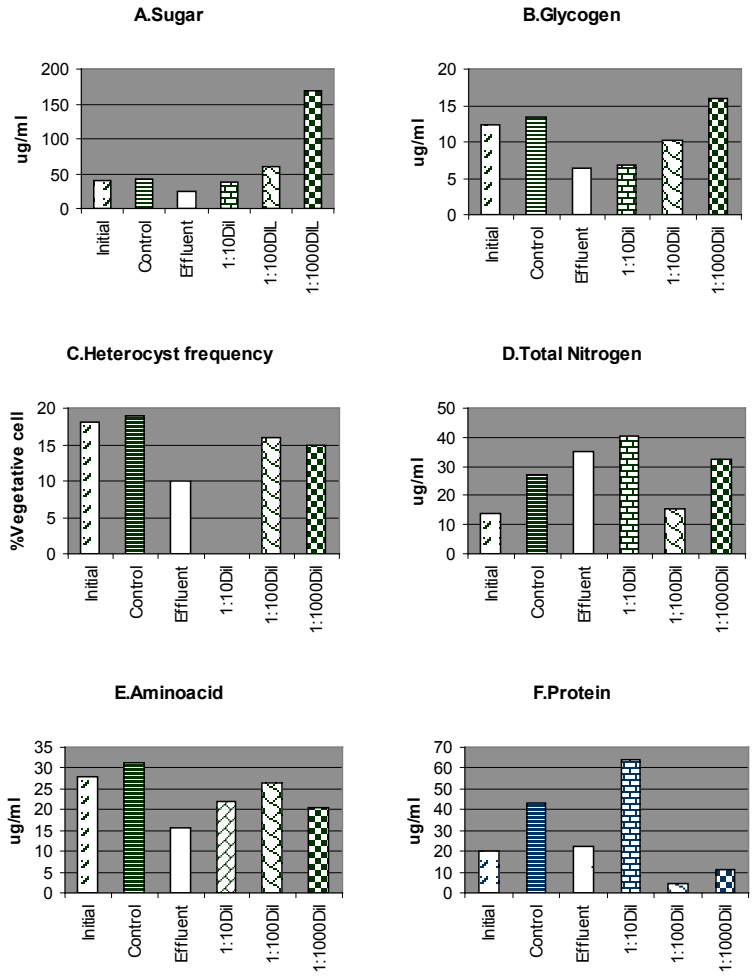

Figure 3: Intracellular leval of Sugar (A), Glycogen (B), Total Nitrogen (D), Aminoacid (E), Protein (F) , contents and Heterocyst frequency (C) of *Anabaena flos-aquae* cultures exposed to tannery effluent treatments.

the changes in heterocyst frequency must be associated with the total nitrogen content of cells. Hence, the total nitrogen content was also checked up during the study. There was no correlation between heterocyst frequency and the intracellular nitrogen content of cultures. As shown in Figure 3D, the cultures continued to maintain high total nitrogen content, when grown in normal N-free medium. It remained significantly high over control cultures after treatment with tannery effluent. Supply of all the dilutions of effluent, enhanced the total nitrogen content of cultures. It is inferred that the supply of effluent in various dilutions such as 1:10, 1:100 and 1:1000 maybe good for the growth of the cultures as revealed by the continued increase in total nitrogen content of cells. The quantitative analysis of aminoacids on the initial and 7th day of treatment was also followed and reported in Figure 3E. The total amino acid content of cells remained at a lower level, when the cultures of *Anabaena flos-aquae* were grown completely effluent. But supply of effluent in diluted form prevented this decrease and their amino acid contents were comparable to that of initial. Amino acids were however, continued to be synthesized by *Anabaena flos-aquae* under normal growth conditions.

The quantitative estimations of total protein (Figure 3F) reveals that it decreased drastically on the 7th day of treatment when the cultures were grown 1:100 and 1:1000 dilutions of tannery effluent. When effluent was diluted 10 times with the sterile medium and supplied to the cultures of *Anabaena flos-aquae* enhanced protein levels of cells even over control cultures.

The filamentous cyanobacterium, *Anabaena flosaquae* was able to grow and its biochemical constituents increased when tannery effluent was provided at 1:10 dilution. It is inferred that the available elements in the tannery effluent are utilized at this concentration for its growth. Studies performed at various laboratories indicate that algae and blue green algae could serve as effective biosorbant for removing heavy metals from contaminated waters [14,15]. They have the capacity to actively transport the metals in to the cells [16]. The ability of *Anabaena flosaquae* in tannery effluent may be used as a strategy to treat pollutants in tannery wastewater. The present study is of preliminary nature checking the ability of *Anabaena flos-aquae* to grow in effluent at various dilutions. The exact mechanism by which *Anabaena flosaquae*` removes pollutant will be investigated in our subsequent studies. Detailed studies will be attempted to understand the mechanism of tolerance of *Anabaena flos-aquae* and check its suitability as a bioremediator.

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