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Short Note

The Photosynthetic Behaviour and Biomass as Indicators for the Resistance and Tolerance Capacity of the Algae as well as Its Potential Use for Tannin Removal in the Tannery Effluents

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Abstract: Tannery effluents have reutilization potentials and may be harnessed for human welfare. Tannin, the chief component of tannery effluent, increases biological oxygen demand. Admittedly disposal of these untreated effluents will affect all life. Such disposal operations will ultimately depend on the biodegradation. Tannins, the chief component of tannery effluents, are degraded by bacteria, yeasts and fungi. Little work has been attempted using blue green algae. It is seen that the algae when made to grow in the effluent (diluted) survives and later flourishes. Since the algae are significant photosynthetic organisms, it is important to investigate the effect of tannins on the pigmentation and also on biomass which serve as bioindicators for tolerance to tannins. The photosynthetic pigments and biomass of the algae were analysed before and after the treatment with effluent. The present investigations showed that cyanobacteria can serve as the potential bioremedial organism for industrial pollution. Today, bioremediation is widely applied in the treatment of contaminated water, soil, sludge and sediments. Bioremediation is the best method for remediation of the long chain molecular organic compounds, hazardous waste and toxicity chemical.

Key words: Tannery, pigments, cyanobacteria, effluent.

Introduction

Water being a major natural resource when being polluted causes deleterious effect on the aquatic flora and fauna and also decreases crop yield. This in turn upsets the ecological balance. Hence more attention was focused towards curbing the activities leading to discharge of any untreated effluents from industries. Among many industries, tanneries occupy a major position. Tanning industries are one of the main economic activities in India. Little more than 2,00,000 tanneries process 55 million hides per year. It has been well documented that wastewater discharged from tanneries without appropriate treatment results in detrimental effects on the ecosystem. The environment is under increasing pressure from solid and liquid wastes emanating from the leather industry. These are inevitable by-products of the leather manufacturing process and cause significant pollution unless treated in some way prior to discharge. Again among all the industrial wastes, those released from tanneries have the highest concentration of pollutants (Nazmul Islam et al., 2011).

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 (Durai et al., 2010). Water bodies receiving the tannery effluent show high BOD, COD and chloride levels that are well above the stipulated concentrations prescribed by the Indian Standard Institute (ISI). Where effluent is

discharged direct into streams and rivers, it needs to be of higher quality as the environment is sensitive and highly susceptible to damage. Tannery effluents have reutilization potentials and may be harnessed for human welfare. Admittedly disposal of these untreated effluents will affect all life. Such disposal operations will ultimately depend on the biodegradation. Though many conventional physicochemical methods of effluent treatment 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 microbes especially cyanobacteria. The treated effluents from tannery industry are collected and added to the cyanobacterial growth medium in various proportions. The photosynthetic pigments, biomass and nitrogen status of BGA were analyzed before and after the treatment with effluent. '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. The major advantage of this technology is that the concentrations of pollutants in the polluted environment are reduced to a very low level using inexpensive biosorbent materials. In the present study, photosynthetic nitrogen-fixing cyanobacterium are used to find out their efficacy in remediating the tannery effluent. It will be 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.

Experiment

Culture

The stock cultures of *Anabaena* sp. and *Westiellopsis* sp., would be maintained in BG11 medium (Stanier, 1971). The inoculated flasks will be kept in a light chamber at 25 ± 1ºC and at 3000 lux intensity for 13 h/day and 11 h/day in dark.

Chemicals

The tannic acid used was from E.Merck India Ltd. The effect of tannic acid on cyanobacteria was studied by inoculating algal cultures in different concentrations of tannic acid.

Treatment

Log phase cultures of BGA were used for the treatment. They were inoculated in the N-free BG-11 medium (Control) with or without the tannery effluent (Treated) to get a final chlorophyll concentration of cultures at 5 µg Chl/ml. Cultures were grown in 500 ml conical flasks and kept in 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 Arnon (1949) as modified by Mckinney's procedure (Mckinney, 1941). The estimation of Cartenoids was performed by Myers and Kratz (1955). Phycobiliprotein (Bennett and Bogorad, 1973), heterocyst frequency (Fogg, 1944), photosynthetic oxygen evolution and respiratory oxygen consumption were also evaluated in the samples.

Biomass Evaluation

Total free amino acids and total sugars were determined in the samples using Jayaraman (1985). Total protein was determined by the method of Lowery et al. (Humphries, 1956). The estimation of total nitrogen was performed according to Humphries.

Results

Tannic acid degradation by cyanobacteria was attempted by using tannic acid at three different concentrations as 10, 20 and 50 µg/ml of the medium. One ml of uniform algal suspension from logarithmic phase of growth was used as an inoculum and the inoculated flasks were kept in a light chamber. The algal cells were immobilized using sodium alginate and the gels were used for tannic acid degradation in BG 11 medium.

The chlorophyll content was estimated on the 0-day, 15th day, 25th day and 40th day respectively. It has been observed that cells grown in tannic acid containing medium showed a higher growth rate when compared to the growth medium. The tannic acid was incorporated in the medium in different concentrations as 10 µg/ml, 20 µg/ml and 50 µg/ml respectively. After a week, growth was estimated in terms of chlorophyll content. The

results showed that 50 µg/ml of tannic acid supported better growth of algal cultures. Among the two cultures, *Anabaena* showed better growth than *Westiellopsis* sp. (Figure 1). Similar trend was also seen in carotenoids as shown in Table 1. Table 2 shows variation in the accessory pigments as phycocyanin, allophycocyanin and phycoerythrin. On 25th day, the phycocyanin was seen to be increased in *Anabaena* sp. from 0.65 µg/ml of 0-day to 0.97 µg/ml, which was higher than the control, which was 0.91 µg/ml on the 25th day. However, a decrease was observed in *Westiellopsis* sp. Both *Anabaena* and *Westiellopsis* sp. were found to have a decrease in allophycocyanin content.

Table 1: Carotenoid content (µg/ml) in blue green algae grown in tannic acid (50 µg/ml)

Culture	*0-Day*	*15TH Day*	*25TH Day*
Anabaena control	13	20	24
Anabaena + tannic acid	21	50	56
Westiellopsis control	23	80	82
Westiellopsis + T. acid	20	60	67

Regarding phycoerythrin content both *Anabaena* and *Westiellopsis* sp. were seen to have a reduced content in medium with tannic acid. Regarding protein content, increase was seen in *Anabaena* sp. from 25th day, but on the 40th day, increase recorded was 100 µg/ml

Table 2: Accessory pigments content (µg/ml) of blue green algae in tannic acid (50 µg/ml) containing media

	Culture	*0-Day*	*15th Day*	*25th Day*
Phycocyanin	Anabaena control	0.7	0.85	0.91
	Anabaena+ tannic acid	0.65	0.86	0.97
	Westiellopsis control	1.23	1.66	1.75
	Westiellopsis + T. acid	1.21	1.28	1.3
Allophycocyanin	Anabaena control	0.73	0.8	0.9
	Anabaena + tannic acid	0.54	0.6	0.65
	Westiellopsis control	0.70	1.11	1.3
	Westiellopsis+T.acid	0.71	0.76	0.7
Phycoerythrin	Anabaena control	0.69	0.71	0.76
	Anabaena + tannic acid	0.5	0.053	0.21
	Westiellopsis control	0.77	0.97	1.2
	Westiellopsis + T. acid	0.65	0.071	0.09

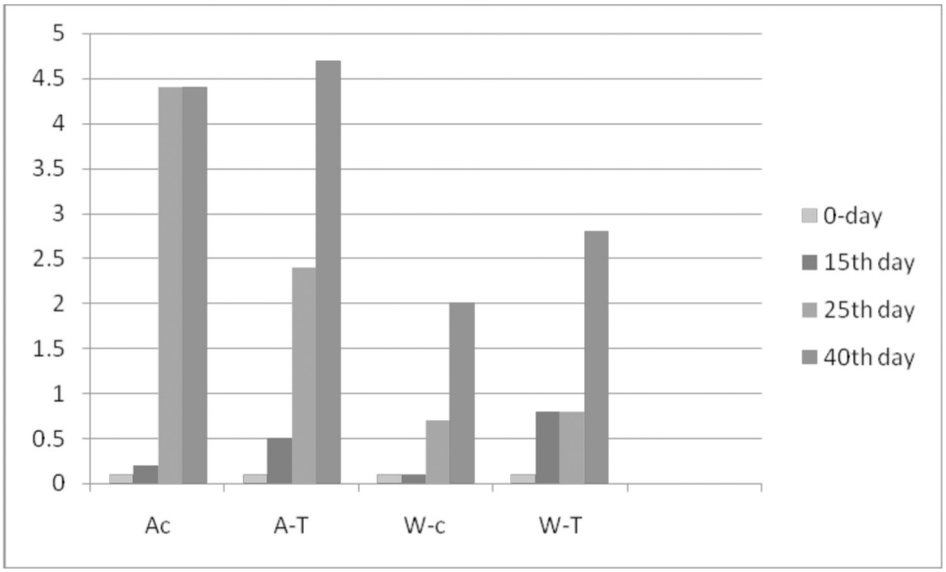

Figure 1: Chlorophyll content in blue green algae grown in tannic acid (50 µg/ml).

from 38 µg/ml as shown in Figure 3. Regarding the carbohydrate content, it was seen that both *Anabaena* sp. and *Westiellopsis* sp. showed marked increase. When compared, *Westiellopsis* sp. was more efficient since the increase was from 12 µg/ml to 43 µg/ml on 15th day, however *Anabaena* sp. also showed increase from 4 µg/ml to 32 µg/ml on 15th day (Figure 2). Marked difference was not observed in lipid content in *Anabaena* sp., while a slight increase was seen in *Westiellopsis* sp.

The nitrogen content corresponded to the protein content, showing a marked increase (Table 3). The nitrogen content can be documented with the number of heterocysts. The percentage of heterocysts was seen to increase from 8 in control to 15 in tannic acid treated cells of *Anabaena* sp. However, there was only a slight increase as from 7% to 8% in *Westiellopsis* sp. (Table 4).

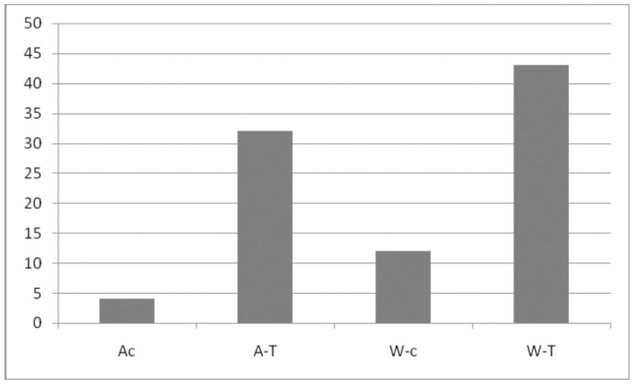

Figure 2: Carbohydrate content (µg/ml) in blue green algae grown in tannic acid (50 µg/ml).

Table 3: Nitrogen content (µg/ml) in blue green algae grown in tannic acid (50 µg/ml)

Culture	*0-Day*	*15TH Day*	*25TH Day*
Anabaena control	12	28	33.6
Anabaena + tannic acid	5	0	112
Westiellopsis control	5.2	5.8	8.0
Westiellopsis + T. acid	5.1	8.16	32

Table 4: Heterocyst % blue green algae grown in tannic acid (50 µg/ml)

Culture	*Vegetative*	*Heterocyst*	*% of heterocyst*
Anabaena control	20	1.6	8
Anabaena + tannic acid	10.6	1.6	15
Westiellopsis control	21.3	1.6	7
Westiellopsis + T. acid	21.3	1.8	8

Discussion

El-Bestawy (2008) confirmed the advantageous potential of using the tested cyanobacterial species for the treatment of contaminated wastewater. Results also clearly showed the quality improvement of the discharged wastewater which in turn will eliminate or at least minimize the expected deterioration of the receiving environment. The photosynthetic pigments of cyanobacteria are located in thylakoids that lie free in the cytoplasm near the cell periphery. Cell colours vary from blue-green to violet-red. The green of chlorophyll-*a* is usually masked by carotenoids (e.g. beta-carotene) and accessory pigments such as

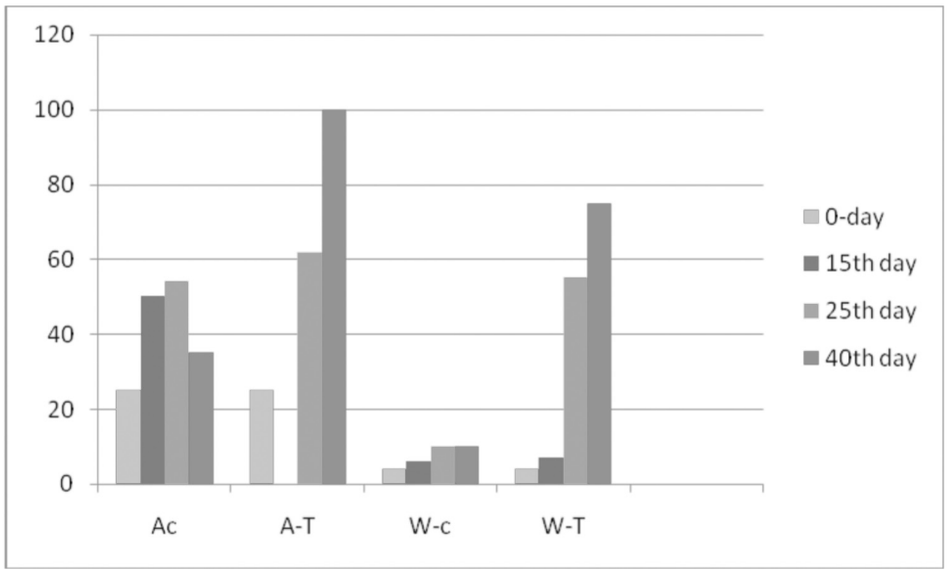

Figure 3: Protein content (µg/ml) in blue green algae grown in tannic acid (50 µg/ml).

phycocyanin, allophycocyanin and phycoerythrin (phycobiliproteins). The pigments are embodied in phycobilisomes, which are found in rows on the outer surface of the thylakoids (Douglas, 1994). All cyanobacteria contain chlorophyll-*a* and phycocyanin and are prolific producers of natural products (Kehr et al., 2011). The basic features of photosynthesis in cyanobacteria have been well described (Ormerod, 1992).

Cyanobacteria are oxygenic phototrophs possessing two kinds of reaction centres, PS I and PS II, in their photosynthetic apparatus. With the accessory pigments mentioned above, they are able to use effectively that region of the light spectrum between the absorption peaks of chlorophyll-*a* and the carotenoids. The ability for continuous photosynthetic growth in the presence of oxygen, together with having water as their electron donor for CO_2 reduction, enables cyanobacteria to colonise a wide range of ecological niches (Whitton, 1992). Phycobiliprotein synthesis is particularly susceptible to environmental influences, especially light quality. Chromatic adaptation is largely attributable to a change in the ratio between phycocyanin and phycoerythrin in the phycobilisomes. Thus, cyanobacteria are able to produce the accessory pigment needed to absorb light most efficiently in the habitat in which they are present. Such being the significance of the primary and accessory pigments, it is rather important to note that there is increase in the pigmentation of the chosen isolates when grown in tannic acid containing media. This is good news, since such photosynthetic autotrophs can be allowed to grow in tannery effluents and may efficiently photosynthesise and also serve as bioremediators.

Cyanobacteria have a remarkable ability to store essential nutrients and metabolites within their cytoplasm. Prominent cytoplasmic inclusions for this purpose can be seen with the electron microscope (e.g. glycogen granules, lipid globules, cyanophycin granules, polyphosphate bodies, carboxysomes) (Fay and Van Baalen, 1987). Reserve products are accumulated under conditions of an excess supply of particular nutrients. For example, when the synthesis of nitrogenous cell constituents is halted because of an absence of a usable nitrogen source, the primary products of photosynthesis are channelled towards the synthesis and accumulation of glycogen and lipids. Also the increase in the heterocysts under the influence of tannins, indicate that nitrogen fixation is enhanced by tannins. Whether tannins have an effect on nif genes has not been studied. Investigations are undertaken to study the exact *mechanism* involved in tannin degradation, and how these isolates serve as bioremediators.

Conclusion

The use of micro algae for the treatment of tannery effluent has been subject of research and development for several decades. Tannins, the chief component of tannery effluents, are degraded by bacteria, yeasts and fungi. Although, degradation of tannins is widely reported in the literature, reports describing tannin degradation by cyanobacteria are very scarce. Since these cyanobacteria are photosynthetic autotrophs, current study reveals the increase in photosynthetic pigments in the presence of tannins. The increase was observed in primary and accessory pigments, proving that cyanobacteria are efficient bioremediators. In addition to its environmental importance, the organism may also be useful as a microbial cell for effective conversion of wastes in tannery effluent to wealth. It also helps to enrich the soil by fixing atmospheric nitrogen and can serve as biofertilizers as well.

References

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase from *Beta vulgaris*. *Plant Physiol*, **24:** 1-15.

Douglas, S.E. (1994). Chloroplast origins and evolution. *In:* D.A. Bryant (ed.), The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht.

Durai, G., Rajamohan, N., Karthikeyan, C. and M. Rajasimman (2010). Kinetics Studies on Biological Treatment of Tannery Wastewater Using Mixed Culture. *Int J Chem Biol Eng*, **3:** 105-109.

El-Bestawy, E. (2008). Treatment of mixed domestic-industrial wastewater using cyanobacteria. *J Ind Microbiol Biotechnol*, **35(11):** 1503-1516.

Fay, P. and C. Van Baalen (1987). The Cyanobacteria. Elsevier, Amsterdam.

Fogg, G.E. (1944). Growth and heterocyst production in *Anabaena cylindrica* Lemm. *New Phytol*, **43:** 164-175.

Humphries, E.C. (1956). Mineral components and ash analysis. *In:* Modern Methods of Plant Analysis. **1:** 469-502. Peach, K. and Tracey, M.V. (eds).

Jayaraman, J. (1985). Laboratory Manual in Biochemistry. Wiley Eastern Ltd., Chennai, India.

Kehr, J.C., Gatte Picchi, D. and E. Dittmann (2011). Natural product biosyntheses in cyanobacteria: A treasure trove of unique enzymes. *Beilstein J Org Chem*., **7:** 1622-1635.

Mckinney, G. (1941). Absorption of light by chlorophyll solution. *J Boil Chem*, **140:** 315-322.

Myers, J. and W.A. Kratz (1955). Relations between pigment content and photosynthetic characteristics in a blue green alga. *J Gen Physiol*, **39:** 11-22.

Nazmul Islam, K.M., Khaled Misbahuzzaman, Ahmed Kamruzzaman Majumder and Milan Chakrabarty (2011). Efficiency of different coagulants combination for the treatment of tannery effluents: A case study of Bangladesh. *Afr J En Sci Tech*, **5:** 409-419.

Whitton, B.A. (1992). Diversity, ecology and taxonomy of the cyanobacteria. *In:* N.H. Mann and N.G. Carr (eds) Photosynthetic Prokaryotes. Plenum Press, New York.