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METABOLIC RESPONSE AND NUTRIENT REMOVAL BY *TOLYPOTHRIX TENUIS* (KUTZ.) FROM FERTILIZER INDUSTRIAL EFFLUENT

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ABSTRACT

With an increase in industrialization the problem of effluent disposal and maintaining the quality of natural ecosystem is becoming acute. Replacing the conventional approach of disposal or dilution, new technologies of bioremediation, detoxification, accumulation, and disinfection of the system are being developed. Exploitation of cyanobacteria in bioremediation is one of the recent trends. In the present study, cyanobacteria -*Tolypothrix tenuis* (Kutz.) was treated with fertilizer industry effluent to find out its nutrient removal efficiency and also metabolic changes occurring due to it. The metabolic changes (Growth- Chlorophyll-a, Carotenoids, Proteins, Carbohydrate, Phenols and Amino acids) and nutrient removal (PO_4, SO_4 & NO_2 uptake) were determined after every three-day interval for 15 days. Results showed growth of *T. tenuis* in 90 per cent effluent concentration was inhibited by 65% that compared to control at the end of 15 days. The test concentrations caused a concentration dependent decrease in pigment content. A decrease in carbohydrate values was observed with rise in effluent concentrations. However, Phenols were found to increase with increased effluent concentrations but amino acids were found to decline. Rise in removal of phosphate by the organism was observed in all the doses of effluent in medium. Removal of nutrients from the medium was found to increase.

INTRODUCTION

In wake of growing industrial civilization, the problems of effluent disposal and maintaining the quality of natural ecosystem are becoming acute. The need to recycle waste has led to the development of new technologies that emphasize detoxification, accumulation and disinfection of the system. Use of oxygen evolving organism is one of the recent trends in bioremediation. Cyanobacteria are one of the most diverse groups of oxygenic photosynthetic prokaryotes exhibiting versatile physiology and wide ecological tolerance that contributes to their success over a vast range of environment.

Cyanobacteria are widely used for purification of polluted water and assessment of water quality

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.Untreated or partly treated industrial effluents which are discharged into water, deteriorate water quality and such water bodies receiving industrial effluents are highly toxic for growth of the phytoplankton and other organisms inhabiting the system. These industrial effluents may contain various toxic components (Kaur *et al.* 1993 and Rai *et al.* 1999). Cyanobacteria are capable of reducing toxic nutrients out of industrial effluents (Dash and Mishra, 1999) and convert into non-toxic nature if not fully but partly. Therefore, various scientists have emphasized the use of cyanobacteria for wastewater treatment and the metabolic changes (Waghmare *et al.* 1986 & Manoharan and Subramanian, 1992). Rai *et al.* (1999) carried out the changes in biochemical profile of *Anabaena flos-aquae* and *Synechococcus cedrorum* in response to carpet industrial effluent. Kanika Sharma et al (2003) carried out the interactions between cyanobacteria and dairy effluent. Neelam Verma et al (1986) employed Cyanobacteria - *Anbaena torulosa* and *A. cylindrica* to assess the functional and structural changes due to pollution stress from electroplating industrial effluent. The mode of action and metabolic response of cyanobacteria varies from one species to another and also depending upon type of effluent.

Therefore, it is very essential to carry out the response of metabolic changes and nutrient removal from fertilizer industrial effluents. Nirmal Kumar et al (1991, 1996 & 2002) and Rana and Kumar (1995) have extensively investigated the growth, pigments and biochemical response of cyanobacteria in relation to various pesticides along with their combinations. In the present investigation, *Tolypothrix* species has been employed to study the biochemical response and removal of toxic chemical like sulphate, phosphate and nitrate from the fertilizer industrial effluent as these nutrients cause water pollution and eutrophication in surrounding waters .The biochemical changes such as chlorophyll-a, carotenes, total sugars, proteins, amino acids, phenols of *T. tenuis* have been carried out.

MATERIALS AND METHODS

Effluent samples were collected from Gujarat State Fertilizer Chemicals (GSFC) located near Baroda, Gujarat, India as the industry discharges enormous amount of effluent. Effluent samples were analyzed using standard methods (APHA, 1996) for the following properties: sulphate, phosphate and nitrate. The filamentous, heterocystous, nitrogen fixing cyanobacteria, *T. tenuis*, an axenic culture procured from Department of Botany, Madras University, Madras, India was selected for present study and maintained in BG -11 nitrogen free culture media (Rippka *et al.* 1979). Three concentrations 60, 80 and 90 percentage of effluent in N2 free BG-11 media as well as in distilled water were prepared to study efficacy of organism in media as well as water. The effluent doses were added to the culture and made up to 20.0 ml with or without effluent constant. One micro-litre of the exponentially growing *T. tenuis* culture was inoculated in to the medium containing different doses of effluent.

The cultures were grown at 25±2 ^{o}C provided with 3000 Lux light for 14: 10 hrs photo and dark period per day. The experiments were conducted in replicates and repeated twice. Biochemical response and removal of nutrients of N2- fixing *T. tenuis* was observed on every three-day intervals upto fifteen days. The growth of *T. tenuis* was measured in terms of Chlorophyll-a as the biomass component (Arnon, 1957). The biochemical studies included were the estimation of carotenoids (Mackinney, 1941), total carbohydrate (Dubois *et al.* 1956), proteins (Lowery *et al.* 1951), phenols and amino acids (Sadasivam and Manikyam, 1996). The nutrient removal studies were carried out by growing *T. tenuis* in specific nutrient free BG-11 media and estimated phosphates, nitrates and sulphates using standard methods (APHA, 1995).

RESULTS AND DISCUSSION

Chlorophyll-a and carotenoid content of *T. tenuis* to various treatments of GSFC effluent is shown in Figs.1a and 1b. The significant retardation of Chlorophyll-a content was recorded after 12 th day, when treated with increased concentration of effluent in medium as well as water as time progressed, a total decrease of 41 and 64.6 percent in 80 and 90 percentage dose, respectively after 15 days, but the best growth was registered in 60 percentage dose. There after the growth was slow and noticed gradually declined in 90 and 80 percent effluent doses in medium. It could be due to destruction of chloroplast and very well substantiated the results of Manoharan and Subramanian (1992), Arjunan *et al.* (1996). Rai *et al.* (1999) found a significant loss of chlorophyll and disorganization of chloroplast in species like *Oscillatoria*, *Aphanocapsa* and *Anabaena* and *Synechococcus*, when treated with paper mill, petrochemical and carpet industrial effluents, respectively.

CONCLUSION

The maximum increase in weight due to sulphate attack is 2.01% by using untreated tannery effluent, 1.46% by using treated tannery effluent and 1.10% by using potable water specimens, which are very negligible. When 2.0% of concare was added, the maximum increase in weight is for untreated tannery effluent which is only 1.74%. This implies that the sulphate attack is slightly reduced when added with concare admixture.

FROM FERTILIZER INDUSTRIAL EFFLUENT

The maximum increase in weight due to chloride attack is 2.23% by using untreated tannery effluent, 1.40% by using treated tannery effluent and 1.05% by using potable water specimens, which are very negligible. When 2.0% of concare was added, the maximum increase in weight is for untreated tannery effluent which is only 0.16%. Almost the chloride attack for all the specimens casted by using untreated tannery effluent, treated tannery effluent and potable water are almost same ranging from 0.10 to 0.15% which is very much negligible. This implies that the chloride attack is greatly reduced when added with concare admixture.

The loss of weight due to corrosion is 6.01% for M_{30} grade of concrete by using untreated tannery effluent and that of specimen casted by using potable water is only 2.94%. This implies that corrosion is more when untreated tannery water is used. But when 2.0% of concare was added the loss in weight is almost same for the specimens casted by using various waters ranging from 0.09 to 0.13%.

From the various study conducted on the concrete using untreated tannery effluent and treated tannery effluents, the behavior of the concrete is found to be satisfactory. Hence the effluent water can be used for construction purpose by giving a minimal treatment to sulphate attack if necessary.

Similar trend was also observed in Carotene content. However, chlorophyll-a and carotene contents were low in water as compared with effluent doses in medium. There was a gradual reduction in carotenoid content at the end of fifteenth day, a total decrease from 135-µg ml^{-20} cultures to 41 and 27.3-µg ml^{-20} cultures, a reduction of 70 and 80 percent carotene in the 80 and 90 percent doses, respectively. But 60-percentage dose in medium induced carotenoid content of cyanobacteria. Similar observations were recorded by Ramesh Kumar Rai et al (1999), when treated *Anabeana flos–aquae* and *Synechococcus* with

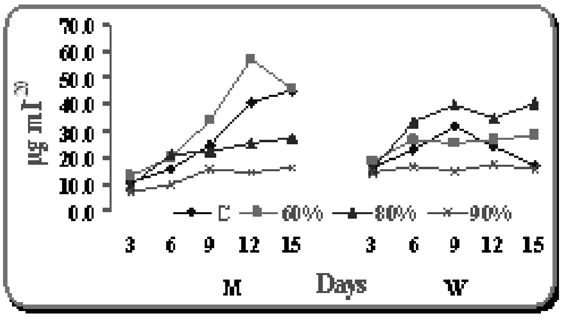

Fig. 1b. Carotenoid content of *T. tenuis* to different doses of GSFC Effluent in Media (M)

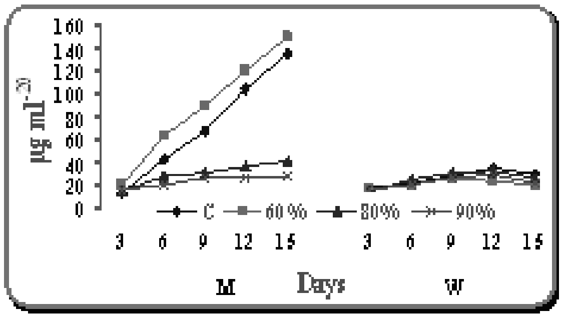

Fig. 1a. Chlorophyll-a content of *T. tenuis* to different doses of GSFC Effluent in Media (M) and water (W)

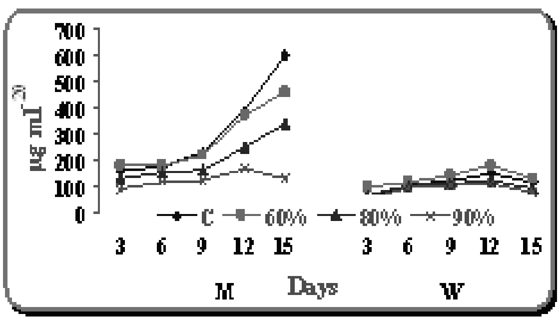

Fig. 2a. Carbohydrate content of *T. tenuis*
doses of GSFC Effluent in Media (M) and Water (W)

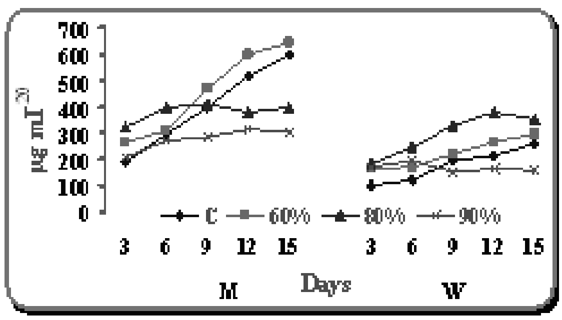

Fig. 2b. Proteincontent of *T. tenuis*
doses of GSFC Effluent in Media (M) and Water (W)

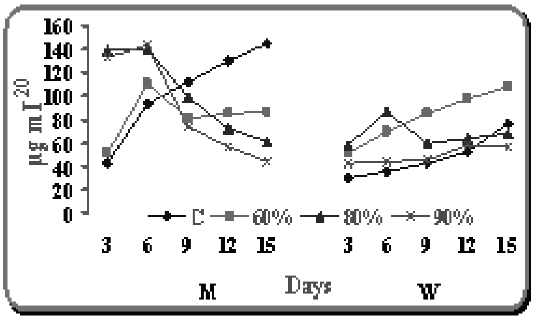

Fig. 2c. Amino Acid content of *T. tenuis* to different doses of GSFC Effluent in media (M) and Water (W)

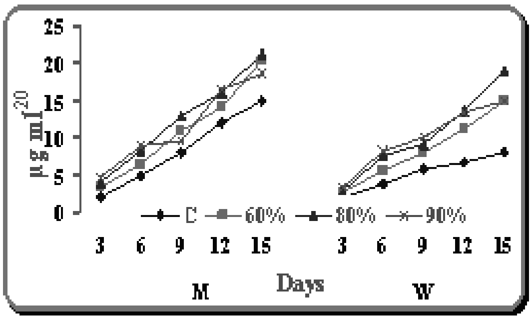

Fig. 2d. Phenol content of *T. tenuis* to different doses of GSFC Effluent in media (M) and Water (W)

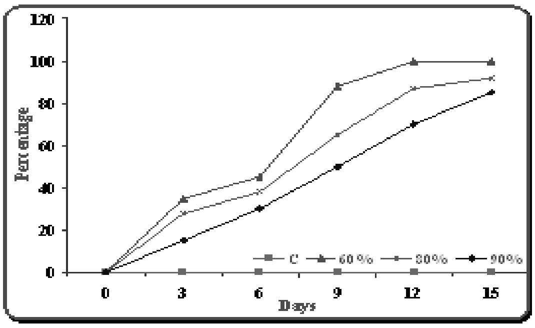

Fig. 3a. % Removal of Phosphate (PO_4) to different doses of GSFC Effluent by *T. tenuis* in Media (M)

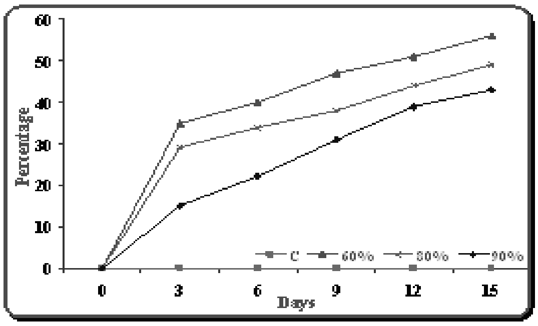

Fig. 3b. % Removal of Sulphate (SO_4) to different doses of GSFC Effluent by *T. tenuis* in Media (M)

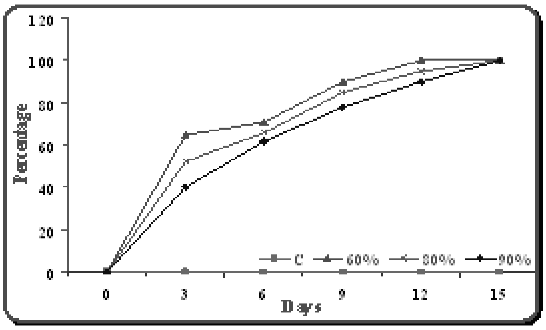

Fig. 3c. % Removal of Nitrate (NO3) to different doses of GSFC Effluent by *T. tenuis* in Media (M)

Carpet industrial effluents at 25, 50 and 100 percent doses.

Biochemical response of *T. tenuis* to different doses of Fertilizer effluents is shown in Figs.2a-d.The variations caused in the physico-chemical activities of *T. tenuis* by fertilizer effluent had its impact on the biochemical composition of the organism. The total carbohydrate content of *T. tenuis* of fertilizer effluent doses in water showed less than that of control but carbohydrate content was found increase upto sixth day in 60 percent dose in medium compared to control, after that, a gradual fall of carbohydrate content was noticed .The total carbohydrate was decreased from 599 to 130 µg ml^{-20} culture, a decrease of 79.9 percent in 90 percent dose by fifteenth day. The higher doses of effluents reduced the sugar contents of *T.* tenuis. Padhy (1985) using diversified species from six genera of unicellular algae proved that chemicals used suppressed the carbohydrate content. This could be the reason in the present investigation that carbohydrate content declined in high doses of effluent treatments and interfered with the photosynthesis process of *T. tenuis*.

The protein content was increased, an increase of

40, 72 and 12 percent in 60, 80 and 90 percent treatments, respectively by 3rd day, perhaps this could be due to partia1 conversion of sugar molecules into proteins at early stage of treatments (Wright, 1978). However, protein content was found to be declined by 33 and 49 percent in 80 and 90 percent doses in medium. Environmental stress induced modifications of protein synthesis have already been reported (Bhagwat and Apte, 1989). Our findings are well substantiated the observations of Rai *et al*. (1999).

The stimulation of amino acids - basic blocks of proteins, was observed in all applied effluent treatments up to 9th day. Like proteins, amino acid content was sharply reduced with increasing concentrations in medium as well as in water. Amino acid content was declined from 144 µg mL^{-20} culture to 86, 61 and 44 µg mL^{-20} culture a decrease of 60, 58 and 70 percent in 60, 80 and 90 percentage treatments, respectively.

Phenols are important aromatic metabolites formed during stress conditions which trigger the various biochemical proposes of the organisms. Initially, phenol content was noticed to be increased in applied treatments as compared to control, both in medium and in water, however, phenol content was found to decline in 90 percent dose of the fertilizer effluent as compared to 60 and 80 percent doses, respectively.

Nutrient Removal

Removal of phosphate, sulphate and nitrate by *T. tenuis* when treated with 60, 80 and 90 percent doses of fertilizer industry is shown in Fig. 3a. Phosphorous, one of the growth-limiting factors is generally present in the form of Phosphate. The cyanobacteria *T. tenuis* was grown in phosphate free BG-11 medium and the removal was registered at every three-day intervals. Phosphate removal was increased with decreased concentrations. The removal of phosphate was observed high amount at 60 percent followed by 80 and 90 percent treatments, it could be perhaps due to increased growth of alga in lower treatment up to ninth day. Phosphate content of effluent was removed 100, 92 and 85 percent in 60, 80 and 90 percent doses, respectively, after fifteenth day.

Sulphates are generally considered to be one of the major pollutants in fertilizer effluents, which are difficult to remove, by conventional treatments. Removal of sulphate by *T. tenuis* at different doses is shown in Fig.3b.The bacteria were grown in sulphate free GB-11 medium. Sulphate was removed gradually and steadily up to fifteenth day beyond which it became almost stabilized in almost all doses. Sulphate content of effluent was removed 56, 49 and 43 percent in 60, 80 and 90 percent doses, respectively, after fifteen days.

Similarly nitrogen is found in the form of nitrate, which is one of the most vital nutrients for the growth of organism. Nitrate removal of *T. tenuis* from fertilizer industry, treated with different doses is shown in Fig 3c.The *T. tenuis* was grown in nitrogen free BG-11 medium. The removal of nitrogen by cyanobacteria was stimulated with decreased concentration of effluent (60 %) that might be due to increased growth of organism. Higher doses proved to be inhibitory of the growth, which might be the reason in the present that the removal of nutrients from effluents is higher in low treatment 60 percent than other applied doses. Nitrate content of effluent was removed 100, 95 and 90 percent in 60, 80 and 90 percent doses, respectively, after twelfth day.

Tam and Wong (1986) demonstrated the sources of using algal cultures to remove nutrients from waste water rich in nitrogenous and phosphorous compounds. The specific use of cyanobacteria both free and immobilized in the effluent for the removal of different forms of combined nitrogen and phosphorous was also reported (Prolux and Dela Noue, 1988). In the present investigation the nitrate and phosphate levels are slightly higher in 90 percent than 80 and 60 percent doses. There was total removal of nitrate as well as phosphate from 60 percent dose after twelfth day and 80 and 90 percent effluent doses of fertilizer industry after fifteenth day. Similar results were also attributed by Manoharan and Subrmanian (1992) when applied different doses of paper mill industrial effluent on *Ocillatoria pseudogeminata*.

From the above investigations, it may be concluded that the removal of sulphate, phosphate and nitrate contents of fertilizer industry by the *T. tenuis* was more in 60 percent applied dose than other two. On the other hand, nitrate removal was high than phosphate and sulphate removal by the organism used. The fertilizer effluent seems to be toxic to *T. tenuis* and reduced major metabolic activities like pigments, photosynthesis, protein, and amino acid synthesis at 80 and 90 percent treatments, however, phenols are affected less. Similar observations also made by Ahluwalia et al. (1989) using *Scenedesmus sp.* as a test organism. However, 60 percent dose of effluent found to be non-toxic to test cyanobacteria. It may also be concluded that effluent from Gujarat State Fertilizer Company stimulated the growth of nitrogen fixing forms like *T. tenuis* when applied in lower doses. This effluent may be used for growing cyanobacteria to obtain the economically important macro – molecules at the low capital investment and can prove good source of coast effective liquid fertilizer.

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