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Microbial decolorization of Triphenylmethane dyes

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Abstract: *Bioremediation exploiting a variety of microorganisms for the degradation of recalcitrant organics appears as an eco-friendly solution to the problem of environmental pollution. Microbes have been talented by nature with the ability of degrading a wide variety of environmental pollutants competently with minimum amount of sludge production. In this work, we have investigated the decolorization and degradation of a mixture of triphenylmethane dyes, Malachite green, Crystal Violet and Basic Fuchsin by a bacterial consortium separately and in a mixed solution. Decolorization studies were carried out with single dye solution as well as mix dye solution with respect to pH, temperature and initial dye concentration. Decolorized products were analyzed with UV-visible spectroscopy and Thin Layer Chromatography, which indicates the process of decolorization in this case, is biodegradation. The decolorized products were also checked for level of COD, wherefrom it was found that the decolorized products were completely safe for the environment up to a concentration of 100 mg/L.*

Keywords: *Decolorization, Triphenylmethane dyes, Bacterial consortium, Dye mix, Degradation*

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I. Introduction

Water is one of the vital necessities for the continued existence of life on this Earth. The water requirement doubles worldwide every 21 years due to the fast increase in the world's population and the industrial activities and additionally, the irregularity in rainfall in these days [1]. It has been anticipated by United Nation that by 2025, 2.7 billion people will be affected by water shortage [2]. Within the next fifty years, the world urban population is expected to increase from 3.4 to 6.4 billion [3] and this will give stress to greater demands on clean water. It is not only the rising population adding to worldwide water stress, but also, industrialization will lead to greater demands for water, resulting in more permanent water scarcity.

Many industries already consume an abundance of water and pollute water sources that could have been directed to drinking and sanitary purposes. The textile industry exhausts a heavy amount of water, utilizing 80-200 m^3 of water per ton of product and producing 1,650 m^3 of wastewater per day [4 and 5]. Wastewater from the textile industry is one of the most polluting of all industrial effluents [6]. Much of the water that is used in textile industries is not reusable and difficult to treat because it is contaminated with dyes or color pigments which have complex aromatic structures [6 and 7]. Along with the dyes, textile wastewater also contains solids, oil, many complex organics and high quantities of chemical residue from process such as bleaching and dyeing. Intense color of the textile wastewater is the most serious problem of the textile industrial effluents. The discharge from these industries is highly colored as enormous amount of dyes remains unsettled during coloring processes which are subsequently washed out into the effluent [8].

Thus the treatment of this effluent is crucial. The discharge of the contaminated effluent without proper treatment can get mixed with surface and ground water and eventually affect the quality of the drinking water [9]. Furthermore, dye effluent if discharged untreated affects the photosynthesis of aquatic plants by preventing the light to penetrate through water. The oxygen levels are affected and in extreme cases may lead to suffocation of aquatic flora and fauna [10].

Dyes are recalcitrant molecules difficult to degrade biologically. From the treatment point of view, the degradation of dyes has received considerable attention. Conventional wastewater treatment processes are suitable for stabilization of nonxenobiotic compounds whereas these processes do not work well with the xenobiotic compounds [11] . Environmental policy in many of the countries now have made it compulsory to decolorize the dye wastewater prior to release.

Thus as an alternative to conventional wastewater treatment, biological processes are gaining more attention to remove these xenobiotic substances from the environment due to eco-friendly, efficient and low cost involvement.

In this study, the decolorization of three triphenylmethane (TPM) dyes namely Malachite Green (MG), Crystal Violet (CV) and Basic Fuchsin (BF) were studied using bacterial consortium. Triphenylmethane dyes

are used widely [12] in textile industries for dying nylon, polyacrylonitrile modified nylon, wool, silk, and cotton. Some of the triphenylmethane dyes are used as medicine in aquaculture industry and as biological stains. Paper, leather, food and cosmetic industries are also major consumers of triphenylmethane dyes. Although used extensively, most of the members of triphenylmethane are animal carcinogen [11]. Hence there are both environmental as well as human health hazards associated with the bioaccumulation of these dyes in environment.

In this study, the three triphenylmethane dyes, i.e., MG, CV and BF were treated with a bacterial consortium, isolated from dye contaminated wastewater from Jaggubazar, Kolkata. The three dyes were used separately as well as in a single solution to study the decolorization pattern. The decolorization was studied with respect to pH, temperature and initial dye concentration. UV-visible spectroscopy and Thin Layer Chromatography studies of decolorized products were also carries out in order to determine mechanism of decolorization. Finally, the decolorized products were also checked for Chemical Oxygen Demand in order to determine the level of toxicity,as it is known that dyes increase COD and lower down the dissolved oxygen of water bodies which is toxic for aquatic life.

II. Materials And Methods

2.1. Dyes used: Triphenylmethane dyes namely Malachite Green Oxalate (MG), Crystal Violet Chloride (CV) and Basic Fuchcsin (BF) Chloride were procured from Merck, India. These dyes were mixed together in equal proportions to make a stock concentration of 500 mg/L and this was used as a stock of dye mix in our experiments.

2.2. Isolation, acclimatization and selection of the microorganism: Dye contaminated wastewater was collected from a small scale textile industry situated at Jaggubazar, Kolkata, West Bengal and the sample was used for isolating potent strains which can remove the triphenylmethane dyes. 1ml of waste water was serially diluted upto10^{-7}. From each of the dilution, 0.1 ml of aliquot was taken and spread with the help of sterile 'L' shaped spreader on Nutrient agar plates in duplicate. The plates were incubated at 35°C for 48 hr. After incubation, 20 bacterial colonies were chosen randomly depending on various colony morphologies. After growing those in agar plates all the microbial isolates were examined for their capability of tolerating the dyes. They were transferred to mineral salt agar medium containing 10 mg/L of three different triphenylmethane dyes separately, in order to determine their tolerance at such concentration. These were then grown in presence of mineral salt broth medium containing triphenylmethane dyes to determine the efficiency of decolorization. The organisms were then acclimatized to higher concentrations of dyes by growing them in presence of gradually increasing concentrations and incubated at 35°C for 24 to 72 hr in mineral salt broth medium containing dyes at 130 rpm. From there 6 different strains were selected for further studies depending on their high level of tolerance to TPM dyes and were used as a bacterial consortium.

Biomass was harvested by centrifugation of the bacterial cells at 8000 rpm for 10 min. After washing with normal saline the required amount of biomass was used for decolorization studies.

2.3. Preparation of medium for growth and decolorization study : Mineral salt medium composition for growth and decolorization studies consist of (g/L): KCl - 0.5, $NaNO_3$ - 2, $MgSO_4$, $7H_20$ - 0.5, $FeSO_4$, $7H_2O$ - 0.01 and MG,CV,BF of varying amounts (0.05-0.5 g/L). All the constituents were weighed accordingly and dissolved in water. The pH of the medium was adjusted to 7 ± 0.2 with either 1(N) hydrochloric acid or 1(N) sodium hydroxide. Then 50 ml of the medium was taken in each of 250 ml Erlenmeyer flask, plugged with cotton and wrapped with brown paper. The entire contents were sterilized in an autoclave at 15 lb/inch^2 pressure for 15 min.

2.4. Batch decolorization process: The experiments were conducted in 250 ml Erlenmeyer flask containing 100 ml of mineral salt medium containing TPM dyes separately as well as in mix solution, which was inoculated with 0.5 ml log phase culture of each bacterial cells in consortium containing approximately 0.8-0.9 ×10^9 cells/ml. To evaluate the effects of various factors on the efficiency of dye decolorization, the batch dye decolorization experiments were carried out at different initial dye concentrations (50 – 500 mg /L), pH (3 – 9) and temperatures (25-50 ^oC). The pH of the media were adjusted with the help of 1N HCl or 1N NaOH. The batch decolorization process was performed under shaking condition (130 rpm). After decolorization of the dye from the media, the dye decolorization was determined by % decolorization of the dye.

2.5. Measurement of % decolorization : After growing the bacterial strains together in dye containing mineral salt medium for 48 hr, the broth was centrifuged at 8000 rpm for 10 min. Clear supernatant was used for determination of dye decolorization in terms of % decolorization. MG, CV and BF in a single solution and separately were scanned from 300-700 nm, where from maximum absorption (λ_{max}) of MG,CV,BF and dye mix were obtained at 617, 590,540 and 585 nm respectively . The % decolorization was calculated using the following expression:

% decolorization = [(initial absorbance-observed absorbance) /initial absorbance] ×100

2.6. Analyses of decolorized products: The decolorized products of mixed TPM dyes were analyzed by UV-visible spectroscopy and Thin Layer Chromatography. The dye decolorization process could be brought about either by biosorption of the dye by the bacterial cells or by degradation of the dye molecules into smaller and simpler compounds. This could be confirmed by UV-visible spectroscopy. If the decolorization was because of biosorption, the original absorption peak would decrease proportionally but if the decolorization was due to the biodegradation then the main peak corresponding to the dye would be either completely lost or a new peak would appear after decolorization [13]. The above mentioned statement can be ascertained once again by analyses of the decolorized products by TLC. If new compounds were generated by the process of biodegradation their presence could be obtained by chromatographic separation. Therefore, the decolorized products were analyzed by UV-Visible spectroscopy and Thin Layer Chromatography.

2.7. UV-visible spectroscopy: In order to determine the whether the TPM dyes were decolorized by biosorption or biodegradation, the UV-visible spectrum of the dye mix and its decolorized products were scanned from 300-800 nm. After complete decolorization , the decolorized medium was centrifuged at 8000 rpm for 10 minutes and supernatant obtained was used to extract metabolites with equal volume of ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of methanol and used for analysis. Degradation of TPM dyes were further examined and confirmed by thin layer chromatography (TLC).

2.8. Thin Layer Chromatography: Metabolite formation was examined by thin layer chromatography (TLC) using commercially available TLC plates coated with silica gel on aluminum foil. The solvent system used was n-propanol: ethyl acetate: acetic acid: distilled water (6:1:1:2 v/v). The ethyl acetate extract was dissolved in methanol and the visualization of separated products was done in UV chamber.

2.9. COD analyses of TPM dyes and its decolorized products: The COD of MG and its decolorized products were determined according to the standard titration method [14].

III. Results and Discussion

3.1. Effect of initial dye concentration on dye decolorization: In order to study the effect of initial concentration on dye decolorization , the experiments were carried out at different dye concentrations ranging from 10-500 mg/L for 48 hr under shaking condition. Figure 1 demonstrates the effects of initial dye concentration on decolorization. The best decolorization was obtained in case of Malachite Green, followed by Basic Fuchsin, Crystal Violet and dye mix. It was found that high degree of decolorization was obtained at lower concentrations , as the initial dye concentration increases the decolorization efficiency dropped down. Khehra et al. (2005) [15] suggested that the decrease in decolorization efficiency might be due to the toxic effect of dyes, on organisms which is an important consideration for its field application. Initial concentration provides an important driving force to overcome all mass transfer resistance of the dye between the aqueous and solid phases [16].

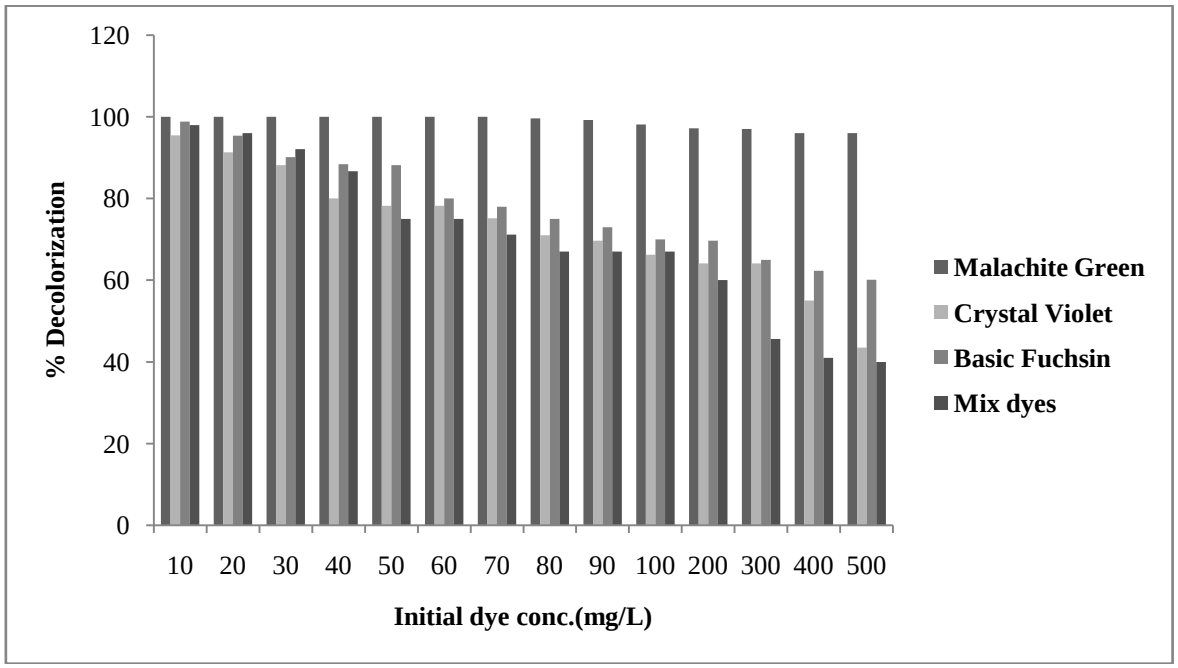

Figure 1: Effect of initial dye concentration on % decolorization of TPM dyes

3.2 Effect of pH on dye decolorization : The pH tolerance is an important consideration for industrial applications because processes are usually performed under alkaline conditions [6]. The experiment was performed in 250 ml Erlenmeyer flasks containing 100 ml mineral salt medium containing dyes separately and in a mix solution where concentration of the dye was 50 mg/L. It was observed that the dye decolorization varied with change in pH of the medium (Figure 2). The decolorization rate peaked around pH 8 in case of Malachite Green , Basic Fuchsin and dye mix. In case of Crystal Violet dye decolorization was highest at pH 6. It is to be noted that increased decolorization efficiency at pH 8,especially for mix dye solution, is an indication that the organisms can be considered as an efficient one for treating dye contaminated waste water, as most industrial dyes are basic in nature. Therefore, these bacterial consortium can be considered as an efficient one for the decolorization of industrial effluent.

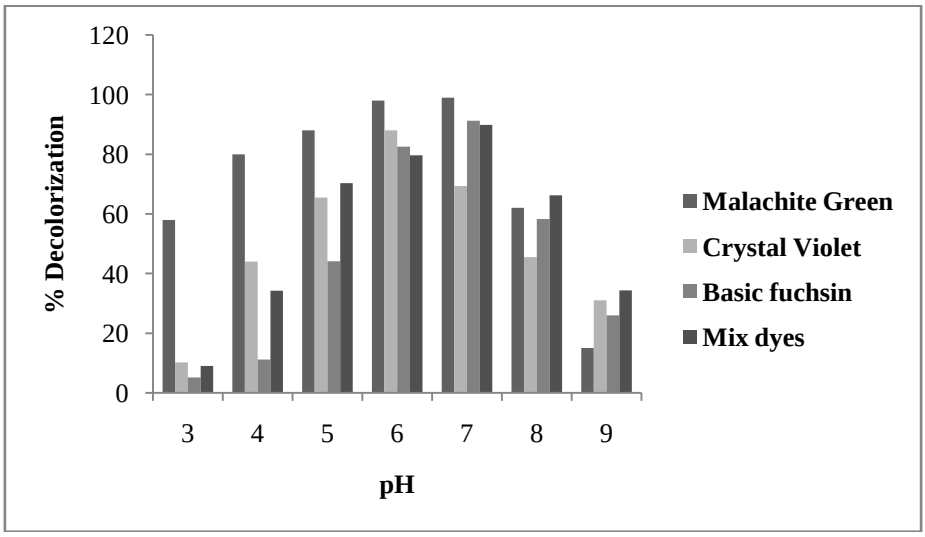

Figure 2 : Effect of pH on % decolorization of TPM dyes

3.3. Effect of temperature on dye decolorization: Determination of temperature requirement of microorganism used for biotechnological purpose is important since temperature requirement above ambient range may require an energy input and hence require input of cost. The microbial consortium can carry on decolorization over a broad temperature range (25-50ºC). The optimum temperature for the decolorization of the dye was found to be in the range of 35-37ºC (88-95 % dye decolorization, Figure 3), which is almost the normal range of temperature in tropical country like India (except for winter). Hence this can be considered as an efficient, cost-effective decolorizer of dye contaminated waste water in these regions. An increase or reduction in temperature above the range may lead to inactivation of the microbial enzymes by denaturation, which, in turn, may slow down the decolorization process.

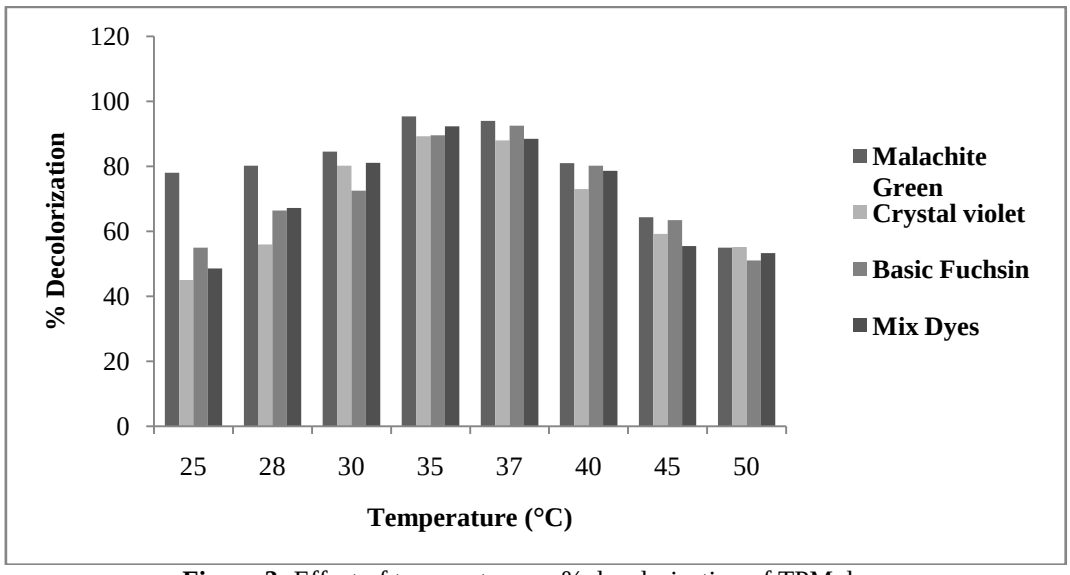

Figure 3: Effect of temperature on % decolorization of TPM dyes

3.4. Results of UV-visible spectroscopy: It was found that the organism exhibited very good consistency in decolorization of the TPM dyes both singly and in mixture. Results of UV-visible scanning analyses (300-700 nm) of the decolorized products of dye mix revealed that the characteristic absorption peak of dye mix of 585 nm was completely lost and two new absorption peaks appeared at 373 and 419 nm (Figure 4) . It was already established [16] that if the decolorization of dye is because of biosorption, the original absorption peak decreases proportionally but if the decolorization is due to the biodegradation then the main peak corresponding to the dye will be completely lost or a new peak will appear after decolorization. Therefore, the process of decolorization in this study indicates biodegradation of the dyes.

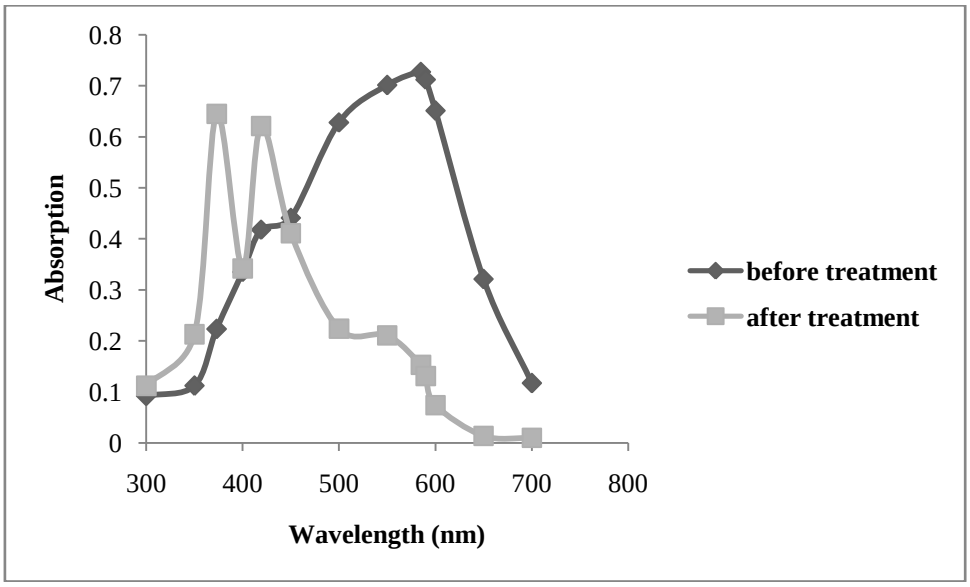

Figure 4: UV-visible study of dye mix before and after microbial treatment

3.5. Results of Thin Layer Chromatography: Thin Layer Chromatographic separation of extracted metabolites confirmed the degradation of TPM dyes. The RF value of TPM mix was noted as 0.0.42 where as extracted metabolites had shown an RF value of 0.31. The RF value is defined as the ratio of the distance travelled by the solute and the distance travelled by the solvent along the chromatographic plate, where both distances were measured from a commom origin. The changes in the movement of dye mix and their decoorized products (Figure 5) , as well as the change in the RF value in this case definitely indicates the biotransformation of TPM dyes into new products, which further confirms the process of biodegradation in this case.

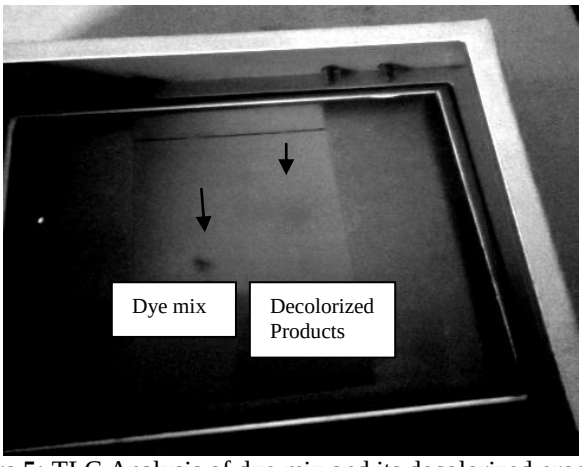

Figure 5: TLC Analysis of dye mix and its decolorized products

3.7. COD analyses of TPM dyes and its decolorized products: The COD values of dye mix with different concentrations TPM dyes and its decolorized products are given in Figure 6. It was found that the treatment of the dyes with the microorganism decreased the COD level of the dyes. However, in this case, upto the concentration 100 mg/L was considered; the COD value of decolorized products ,here, has already reached 280 mg/L, which is even higher than the upper limit of COD for disposal of effluent into surface water sources set

by Indian Standards[18] . Thus the microbial treatment in this experiment, has also lowered down the COD load, which means lowering down the level of toxicity.

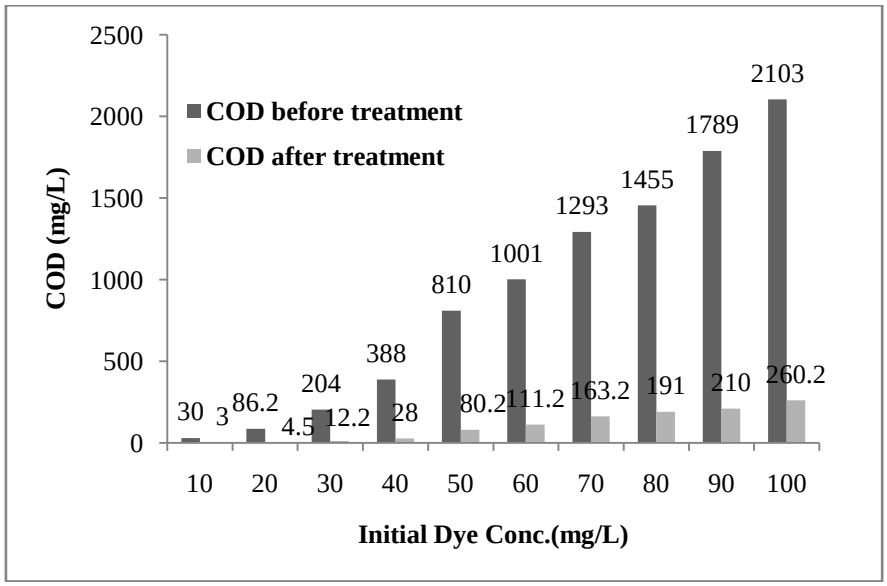

Figure 6: COD of mixed TPM dyes before and after the microbial treatment

IV. Conclusion

Organisms which have a natural habitat in dye contaminated wastewater or dye contaminated lands have natural inherent property of withstanding harmful effects of dyes when grown in presence of them. In this case, we selected a small scale dying industry, collected dye contaminated wastewater from there and isolated some bacterial colonies from that wastewater only which were capable of withstanding high concentrations of TPM dyes. The organisms are yet to be identified, however, their potency as efficient decolorizer of TPM dyes have already been established through this study. The bacterial consortium were capable of decolorizing the dye beyond 500mg/L. The optimum pH for dye decolorization in mxed solution were found to be around pH 8.The optimum temperature was also found to be suitable for using them in tropical country like ours. The mode of decolorization was also found to be biodegradation through UV-visible analysis and TLC study. The decrease in the level of COD also proved the less toxic nature of the degradation products. Therefore, this bacterial consortium can be considered as potential candidate in treatment of real life dye house wastewater after more detailed studies.

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