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Combined Biological and Advanced Oxidation Process for the Treatment of Textile Dye Containing Wastewater

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Abstract- The present study has proven that combined Biological and Advanced Oxidation Process (AOPs) is effective to treat dye-containing wastewater for reuse. With respect to environmental protection and utilization, a two stage treatment process involving adapted bacterial strain and hydroxyl radical (OH*) induced photocatalytic decolorization and degradation of dye-containing wastewater has been studied. The present study was carried out on the decolorization of four commonly used textile azo dyes namely Reactive Yellow 14, Reactive Black 5, Direct Blue 86 and Reactive Red 198, in a successive biological and solar driven advanced oxidation process involving hydrogen peroxide. For biological treatment, a novel autochthonous bacterium *Soil Isolate 04* (SI 04) isolated from wastewater contaminated site have been used. This novel bacterial strain efficiently decolorized three dyes in pH 8, at 37˚C under microaerophilic condition (to a maximum of 90%) in nutrient broth whereas, Direct Blue 86 dye was strongly resistant to decolorization through biological treatment. These dyes were subjected to chemical oxidation involving 10% H_2O_2 under solar energy separately. The result indicates that decolorization efficiency was influenced by operational parameters such as solar light intensity, concentration of H_2O_2, initial dye concentration and time. In combined Biological and Advanced Oxidation Process the decolorization was more efficient (decolorization ranging from 90-100%) for all the four dyes.

Keywords- *Azo dyes, Aeromonas Sps SI 04, H_2O_2, decolorization, photodegradation, hydroxyl radical*

I. INTRODUCTION

Textile industries generate large volumes of wastewater, which leads to a serious problem for the natural ecosystem [1]. The toxic effects of textile industrial wastewater are mainly due to the presence of mixture of dye molecules [2, 3]. It was quantified that more than 80,000 tons/year of dyes are used in textile dyeing process, which needs 70-150 dm^3 of water. It has been reported that the textile industry requires about 40g of dyes for one kilogram of cotton [4]. Azo dyes are one of the largest classes of synthetic dyes, which are widely used in the wet process of the color industry [5]. The presence of one or more azo bonds (-N=N-) and aromatic rings in its chemical structure, it is colored, toxic and resistant to degradation [6]. The release of these dyes without treatment into the environment leads to inimical impact on the aquatic ecosystem as it is toxic to aquatic organisms and are reported as carcinogenic to humans [7]. Therefore, azo dye containing textile wastewaters need to be treated before their discharge into the environment. Numerous physico-chemical and biological methods have been widely used to treat textile wastewater containing azo-dyes [8, 9]. However, the physical and chemical treatments have many drawbacks due to the high cost and release of hazardous sludge as secondary pollution [10]. On the contrary, bioremediation offer a cheaper and more environmentally friendly alternative method for the treatment of textile wastewater [11, 12]. Several studies reported lot of microorganisms such as Bacteria [5], Yeast [13], and Fungi [14] are involved in the subject of color removal; in the mechanism of bioadsorption, biotransformation or degradation.

Although, biological process alone is not enough to remove surfactants, color, and the recalcitrant to levels for direct discharge [15]. To overcome the above mentioned problems with respect to physico-chemical and biological treatments, this study is focused towards the combined use of biological and advanced oxidation process involving hydrogen peroxide. Various reports suggest that the efficiency of wastewater treatment is improved by combining AOPs with biological treatment [16, 17]. Advanced Oxidation Process is achieved by highly oxidizing agent like hydroxyl radical capable of degrading pollutants [18, 19, 20]. The treatment economy could be further improved while using solar energy as radiation instead of UV-light [21, 22].

The main aim of the present study is to evaluate combined biological-advanced oxidation process for complete decolorization of four commonly used textile azo dyes namely Reactive Yellow 14, Reactive Black 5, Direct Blue 86 and Reactive Red 198. The biological treatment is performed by a novel autochthonous bacterium *Soil Isolate 04* (SI 04) isolated from wastewater contaminated site and AOPs achieved by H_2O_2. The influence of H_2O_2 was studied in different concentration. The efficiency of photolysis hydrogen peroxide under solar energy was also evaluated.

II. MATERIALS AND METHODS

A. Dyes and Chemicals

Four different commonly used textile azo dyes were procured from Jamara textile industry, Erode, Tamil Nadu, India. Nutrient broth (Peptone, NaCl, Beef extract and Yeast extract) was obtained from Himedia Pvt. Ltd., Mumbai, India. Hydrogen peroxide was obtained from Merck Pvt. Ltd., Mumbai, India.

B. Isolation, screening and molecular identification of potential dye decolourizing bacteria

The textile effluent polluted soil was aseptically collected from CETP (SIPCOT), Perundurai, Erode, Tamil Nadu, India. One gram of soil sample was added to 100 ml of sterile distilled water and serially diluted involving standard protocol [23]. From each dilution 100 µl of sample was plated on nutrient agar medium and kept for incubation at 37˚C for 24 hrs. Seven bacterial cultures (SK01 to SK07) were isolated based on the color, colony morphology and raised for pure culture on a slant containing nutrient agar for further studies. The organisms were used for decolorization studies after preculturing in nutrient broth (g L^{-1}) at 37 ±2˚C for 16-18 hrs (log phase) under shaking condition at pH 7. All the seven isolates were inoculated individually on a dye (Reactive Red 198 for preliminary study) containing medium and kept for incubation at 37˚C for 72 hrs both in shaking and static conditions. The potential decolourizing strain was subjected to molecular identification through 16s rDNA analysis [24].

C. Optimization of growth conditions

In order to give a suitable condition for their optimal growth a loopful culture was inoculated in sterile nutrient broth and kept for shaking. After reaching 1.0 OD, 5% of inoculum was inoculated in Erlenmeyer flask containing sterile nutrient broth with various pH and kept for incubation at shaking (120 rpm) as well as static condition. The same procedure was followed to optimize temperature.

D. Biodecolorization experiments

The 18 hrs culture (log phase) was incubated with different textile dyes such as Reactive Yellow 14, Reactive Black 5, Direct Blue 86 and Reactive Red 198 at concentration of 100 mg l^{-1}, individually and incubated at 37˚C for shaking and static condition. An aliquot of 5 ml culture media was withdrawn in different time intervals and centrifuged (Eppendorf 5804 R, Germany) at 1000 rpm for 10 minutes to separate biomass. The supernatant was used to confirm decolorization by measuring the change in absorbance in the respective λ_{max} of the respective dyes. All the experiments were performed in three sets. Abiotic control was (without microorganisms) always included. The percentage of the decolorization was calculated as follows [25]:

$$\%Decolorization = \frac{Initial\ absorbance - Final\ absorbance}{Initial\ absorbance} X100$$

E. Advanced Oxidation Process

Advanced Oxidation Process (AOPs) was carried out by using 10% H_2O_2 with 100 mg l^{-1} dye. The sample was irradiated under solar energy. The light intensity was measured by using Lux Meter (Lutron LX-101, Taiwan).

F. Combined treatment of biological and Advanced Oxidation Process (AOPs)

After biological treatment the sample was centrifuged to separate biomass. The microbe free supernatant with 10% hydrogen peroxide was irradiated under solar energy. After the treatment sample was again centrifuged at 10000 rpm for 15 minutes and used for spectral analyzed in order to determine decolorization efficiency.

III. RESULTS AND DISCUSSION

A. Isolation, primary screening and molecular identification of best decolorizer

Seven different bacterial cultures were isolated based on the color, colony morphology and were named as SK01 to SK07. Preliminary screening for identification of the potential dye decolorizing bacteria revealed SK04 as a promising strain by decolorizing Reactive Red198 to a maximum of 89±1.6%. 16s rDNA analysis revealed that SK 04 to be *Aeromonas sps* and the sequence was submitted to the Genbank database under NCBI.

B. Optimization studies

In order to provide an appropriate environment for the best decolorizer to achieve maximum decolorization, optimization study was carried out at various pH, temperature, shaking and static condition. Results revealed that 37˚C under shaking at pH 8 are optimal for the growth of *Aeromonas Sps* SK04 (Fig. 1 and 2).

C. Biodecolorization experiments

As the preliminary experiments revealed decolorizing potential of the novel bacterial strain SI 04, the latter was subjected to decolorize all the chosen four commonly used textile dyes. The decolorization percentage of all the dyes tested is tabulated (Table 1). The selected bacterial isolate SI 04 had the potential to decolorize chemically different dyes at a concentration of 100 mg l^{-1} under static condition at 37˚C, pH 8. The decolorization efficiency was not significant in shaking condition. However, cell growth was poor under static condition as compared to shaking condition. Reports indicate that oxygen is deleterious to the activity of enzyme (azoreductase) which is responsible for azo bond reduction; oxygen also favors bacterial growth under shaking condition [26]. Direct Blue was completely resistant to biological treatment under shaking and at static conditions. Reactive Yellow 14 was decolorized to a maximum of 90% under static condition where as it there was No Decolorization under shaking condition. Ghodake et al. [27] also reported decolorization rate of Direct Brown MR at static by 91.3% and 59.3% under shaking condition. Microorganisms able to grow on dye containing medium under shaking condition, but not decolorize dyes under aerobic condition [27]. Similar kinds of results were reported in the previous studies in case of *Pseudomonas SUK1* [28] and in case of *Pseudomonas aeruginosa* BCH [11, 29]. Our results were in accordance with

the previous reports and indicate that the bacterial cultures under static condition were more efficient in decolorizing dyes used in textile industry.

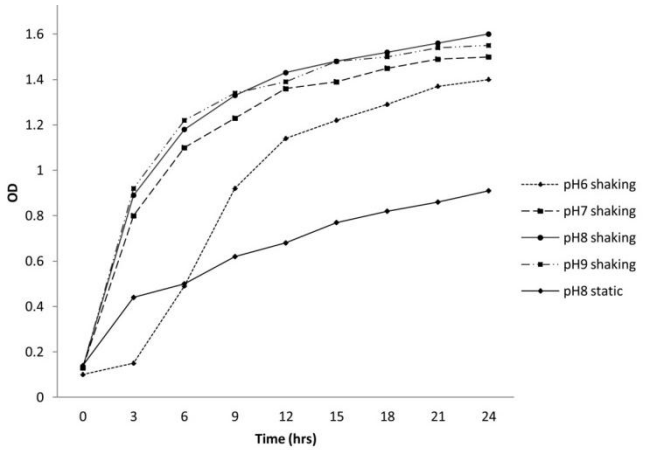

Figure 1. Graph representing the effect of pH and Static/Shaking conditions on the growth of SI 04.

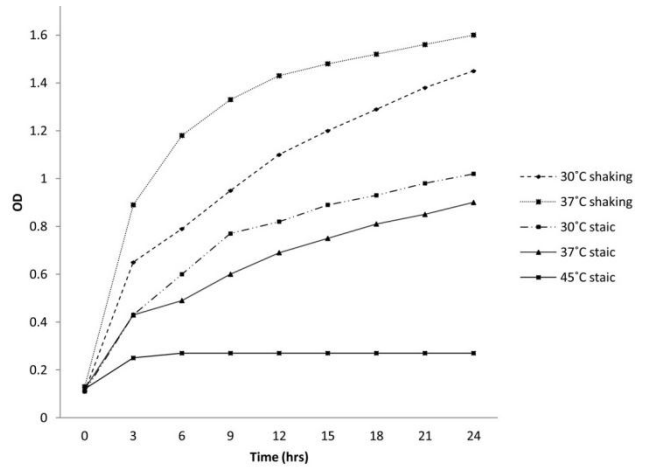

Figure 2. Graph representing the effect of temperature and Static / shaking conditions on the growth of SI 04.

D. Use of Minimal Media

The Decolorizing ability of the bacterial isolate was also tested in minimal media containing the dye at a concentration of 100 mg.l^{-1}. Minimal medium contained (g.l^{-1}): Potassium dihydrogen phosphate 3.0, Disodium hydrogen phosphate 6.0, Ammonium chloride 5.0, Sodium chloride 5.0, Glucose 8.0 and Magnesium sulphate 0.1. The pH was set to 8.0. SI 04 was subjected to decolorization study on all the chosen dyes in minimal media under standardized conditions as described above. None of dyes showed significant decolorization (Data not shown). Due to lack of significant decolorization in under minimal media, further studies were carried out only in nutrient broth.

E. Advanced Oxidation Process (AOPs)

The advanced oxidation process involving H_2O_2 at 10% for 100 ml of dye solution containing 100 mg l^{-1} concentration of dye prepared in 250 ml Erlenmeyer flask with distilled water. It was irradiated under solar energy. Exposure to sunlight was extended until maximum changes in color were observed. Even after 12 hrs of exposure to sunlight the dyes Reactive Black 5, Reactive Red 198 and Reactive Yellow 14 showed insignificant decolorization percentage of 36.87%, 64.80%, 63.07% respectively. Interestingly the dye Direct Blue 86 was completely decolorized (100%) under this condition. Direct Blue 86 was completely resistant to bacterial action (Table 1).

F. Combined biological and sequential AOPs

Biologically decolorized culture broth was centrifuged and 10% of H_2O_2 was added and irradiated under solar energy for 6 hrs. Light intensity was 60000 Lux. The dyes Reactive Black 5, Reactive Red 198, Reactive Yellow 14, and Direct Blue 86 were decolorized completely to a maximum of 100% (Fig 3). Whereas there was an insignificant reduction in the decolorization % of Direct Blue 86 in the two stage process. Punzi et al. [30] reported Bio-sequential Advanced Oxidation Process showed efficient decolorization. The results of the combined treatment process (Table 1) are in accordance with the findings of Rodriguez *et al* (2010) and Parra *et al* (2002) which states that Combined AOPs and biological treatment were able to remove non-biodegradable and toxic pollutants [31, 32]. The biodegradable pollutants are degraded in first stage of biological treatment and non-biodegradable pollutants are degraded in AOP process [33].

TABLE I. DECOLORIZATION PERCENTAGE OF CHOSEN DYES SUBJECTED BIOLOGICAL, AOPS AND BIOLOGICAL SEQUENTIAL AOPS

Sl. No	Name of the Dye	λ_{max} (In nm)	Time taken for decolorization	Biological Treatment (%)		AOP (%)	Bio + AOP (%)
				Shaking	Static		
1	Reactive Black 5	600	24 hrs	72.72±1.91	86.57±2.61	36.80±1.93	100±0
2	Reactive Red 198	517	24 hrs	84.61±1.9	88.75±2.10	64.80±1.06	100±0
3	Reactive Yellow 14	417	24 hrs	ND	90±3.0	63.07±1.72	100±0
4	Direct Blue 86	663	24 hrs	ND	ND	100±0	90.09±0.9

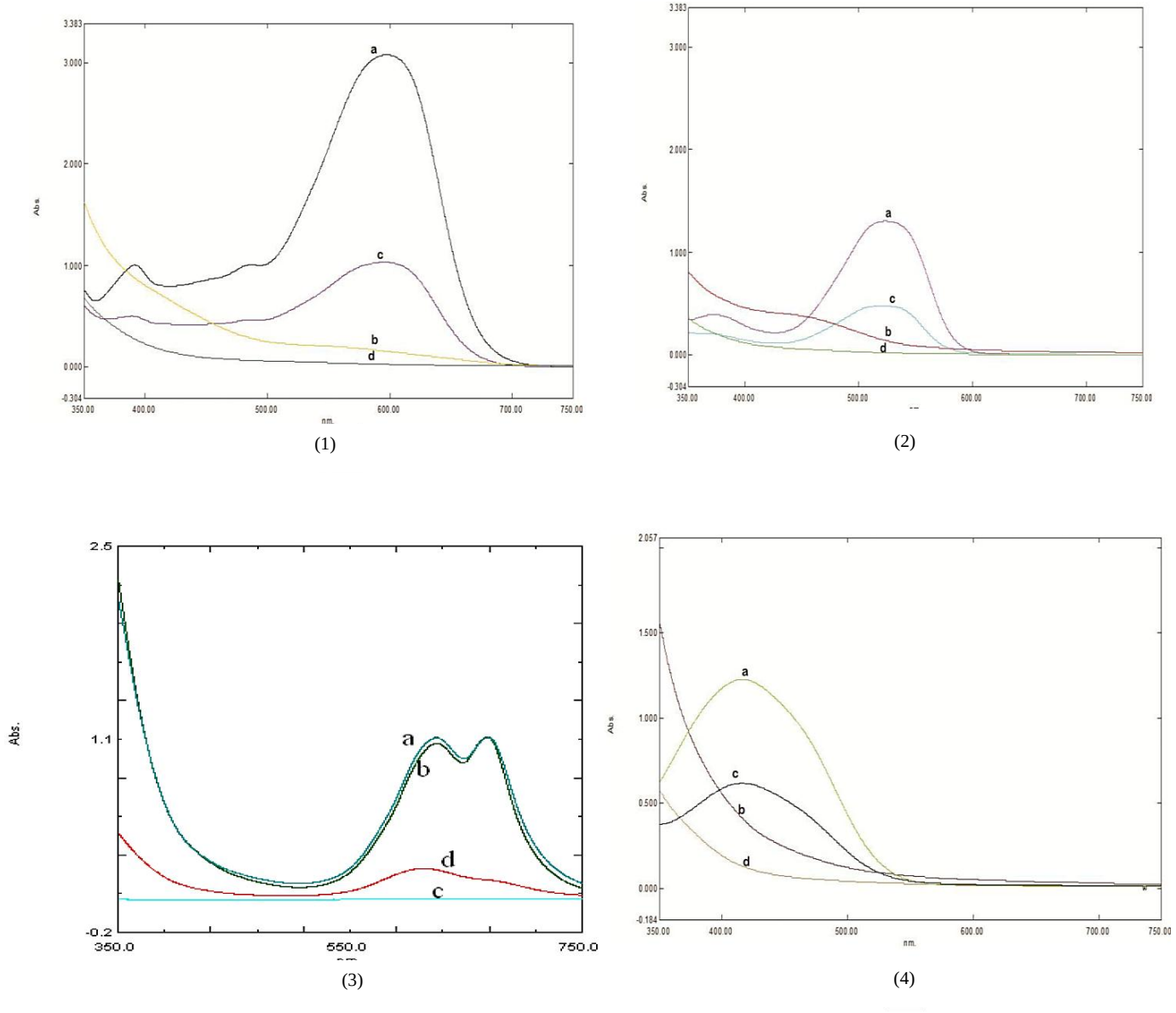

Figure 3. Spectral analysis of [1] Reactive Black 5 [2] Reactive Red 198 [3] Direct Blue 86 [4] Reactive Yellow 14 (a - Abiotic control; b-after biological treatment; c-native AOP; d- biological sequential AOP)

IV. CONCLUSION

Measures to develop environmentally favorable as well as cost effective technique to remove hazardous pollutants from textile waste water has received a great deal of attention. In the present study application of biological, advanced oxidation and combined process was investigated in the treatment of synthetic medium containing dyes (synthetic effluent). Bacteria capable of decolorizing different textile dyes were reported to be isolated from the textile dye – contaminated sites [24]. This is an indication of adaptation of microorganisms to the toxic levels of dyes in the environment. Similarly, in the present study, seven bacterial isolates were raised from the soil samples taken from the contaminated sites. Though most of the bacteria were capable of decolorizing different dyes to different levels, one among them, strain *SI 04* demonstrated significant potential for decolorization. The latter was subjected to molecular identification methods and found to be *Aeromonas sps SK04.* Several authors have reported and identified bacterial strains for such decolorizing potential [25-30]. An interesting finding is in the present study is that, the decolorization for most of the dyes was significant only in static conditions indicating microaerophilic nature of the process involving the novel bacterial culture.

It is claimed that under aerobic condition, there might be a competition between azo dyes and oxygen for reduced electron carriers and also inhibits the Azo-bond reduction activity since

aerobic respiration may dominate utilization of NADH and thus impeding the electron transfer from NADH to Azo bonds [10, 25, 28]. This may be attributed to the maximum decolorization of the chosen dyes in the present study under static conditions. Adaptation of microorganism to broad range of pH and temperature make them more suitable for degradation of dye-pollutants. *SI 04* showed decolorization up to 90 % in the broad pH range 7-9 at 37^{o}C. The broad pH stability in decolorization increases the applicability of this strain since dye waste water is generally of alkaline pH. Various basic and advanced instrumental techniques of chromatography and spectroscopy can be used to isolate and characterize the products of biodegradation of dyes and thus have an insight into the mechanism of biodegradation. To date, very few reports are available on the intermediates or the products of biodegradation of triphenylmethane dyes [17]. The bacterial metabolism of azo dyes is initiated in most cases by a reductive cleavage of the azo bond, which results in the formation of colorless aromatic amines [11].

Although biological degradation methods are one of the most economic processes for wastewater treatment, they are often ineffective to degrade molecules of refractive nature, like those present in textile industry waste waters. Also, the survival of anaerobic biomass in the presence of high concentration of azo dyes is a difficult task. Therefore, for the treatment of this type of waste water other alternative methods have been proposed elsewhere. In the present study, out of four dyes chosen, one dye (Direct Blue 86) was completely resistant to biological treatment and therefore the dye has been further subjected to advanced oxidation process as the second stage of treatment to achieve decolorization. The other dyes which were also significantly decolorized through biological treatment were subjected second stage AOP to enhance decolorization to 100% and to obtain a solution as clear as water. In the advanced oxidation process, UV/H_2O_2 system was employed for the decolorization study. Initially, the dye stability was examined in dark conditions without using H_2O_2 or UV. As the next step, dye stability was examined with H_2O_2 alone in dark condition. No color change was observed. The initial concentrations of H_2O_2 (at 10% v/v) played an important role in the decolorization of dyes. Decrease in the concentration of H_2O_2 increased the time duration for decolorization. In the present study, two-step treatment process namely biological treatment followed by UV/H_2O_2 was also assessed.

In this combined process, all the dyes were decolorized to a maximum of 100%. In the UV-Vis spectral analysis, absorbance peak significantly disappeared in all dyes and real time effluent. To conclude, successful application of biological treatment in the decolorization of highly concentrated synthetic medium was noted. However, few dyes are also noted to be resistant to biological treatment. Therefore, advanced oxidation process involving UV-H_2O_2 was used as the post treatment in which maximum decolorization of 100% was achieved. Our results show that the combined process is more effective than biological treatment or Advanced Oxidation Process independently. The result suggests efficient decolorization and low operational cost can be achieved in the field by Biobased AOPs under solar energy and this combination of techniques is recommended for the textile wastewater treatment.

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