Pollution, 6(3): 503-511, Summer 2020
DOI: 10.22059/poll.2019.288500.675
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Decolorization of Reactive Black-5 High Concentration by Vermicompost Microflora and Detoxification of By-Products by UV-C/H_2O_2 Post-Treatment

Emadi, Z.[1], Sadeghi, M.[1*], Mohammadi-Moghadam, F.[1], Sadeghi, R.[1], Forouzandeh, S.[1] and Sadeghi, R.[2]

1. Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, P.O. Box 8813733435, Shahrekord, Iran
2. Department of Chemical Engineering, Isfahan University of Technology, P.O. Box 8415683111, Isfahan, Iran

Received: 05.09.2019 Accepted: 27.05.2020

ABSTRACT: The presence of synthetic dyes in textile wastewater is a problematic issue for environmentalist. Nowadays, dye removal is practiced via different methods. Among all these methods, biodecolorization is an ideal technique. The present research apples vermicompost microflora to remove reactive black-5 at 35 °C, pH = 7, and under anaerobic condition. At 36h, removal efficiencies of 94.79%, 94.06%, and 93.6% are obtained for concentrations of 800, 850, and 950 mg/ L, respectively. It has also been observed that when the initial concentration rises to 1400 mg/ L, the efficiency drops to 51.57% at 36h. Also, methyl red, methyl orange, eriochrome black-t, and acid blue-113 could be decolorized by the isolated bacterial strain with an efficiency of 94.29%, 92.10%, 90.83%, and 88.95%, respectively. Phytotoxicity Test shows that the parent form of reactive black-5 has not been toxic for the seeds (100% germination for Triticum aestivum and 90% for Maize). When reactive black-5 is treated with isolated bacterial strain under anaerobic condition, none of the seeds remain germinated which might be due to the possible formation of toxic aromatic amines intermediates. Therefore, ultraviolet C + 100 mM H_2O_2 has been used as the post-treatment process for detoxifying of by-products. After the integrated treatment of synthetic wastewater, containing RB-5, complete germination (100%) of Triticum aestivum and Maize is observed. In the post-treatment process, due to the generation and activation of hydroxyl radicals, the toxic aromatic amines compounds convert to the less toxic compounds.

Keywords: azo dyes, anaerobic removal, integrated treatment, phytotoxicity, hydroxyl radicals.

INTRODUCTION

Various industries like textile, food, cosmetic, leather and pharmaceutical are commonly consumed dyestuffs which eventuated in the production of the enormous quantity of dangerous squander (Chaudhari et al., 2013). The textile industry utilizes the colossal quantity of precious fresh water that can cause to produce a huge amount of effluent. Furthermore, this effluent consists of inorganic material like salts, complicated organic materials, bases, and different unutilized dyestuffs (Xu et al., 2016). Approximately more than 7×10^5 ton of synthetic dyestuffs are made each year around the globe (Sadeghi et al., 2019; Chen

* Corresponding Author, Email: sadeghi@skums.ac.ir

et al., 2011). In the midst of all, azo dyes are the biggest group (60-70 %) of the colors and have the most diversity of colors (Deive et al., 2010). The main feature of the azo dyes is the attendance of one or more nitrogen-nitrogen double bonds. These groups bond with the naphthalene and benzene rings, and the functional groups like NH_2 (amino), OH (hydroxyl), COOH (carboxyl), CH_3 (methyl) and Cl (chloro) that cause generation different types of azo dyes (Sudha et al., 2018). Direct discharge of the effluent from dying procedure into the surroundings or wastewater treatment plants due to the unbinding of dyes to the object fibers (around 10-15 %) results in the severe environmental complications (Liu et al., 2017). As stated by described information, for fabrication at an average of 60×10^{4} m of fabric 1.5 million liter/day of wastewater is released into natural water bodies (Nidheesh et al., 2018). Interruption of light entering into the profound layers subsequently abatement of the photosynthesis action, subtraction of dissolved oxygen and damage of biological diversity are the detrimental effects of the discharge of dying effluent in the aquatic ecosystem (Guadie et al., 2017). Because of the formation of dyestuffs from carcinogenic compounds such as benzidine, the dyes are also recognized carcinogen (Daâssi et al., 2013). Unfortunately, one of the applications of textile effluent is for the irrigation of plants, and some of these dyes can create problems for the plants as a result of the poisonous properties of them (Imran et al., 2016). Therefore, elimination of the colors from textile wastewater prior to release into the environment is significant and vital (Ma et al., 2014). Nowadays different Physical, chemical and biological methods are examined for the treatment of the azo dyes from textile wastewater (Tan et al., 2014). Adsorption, membrane filtration, coagulation, photo and electro removal are common methods for the treatment of colored wastewater, but these methods have some disadvantages like the formation of the huge quantity of sludge and overpriced. While on the opposite site, biological methods by fungi and bacteria are considered as cost-effective and businesslike methods (Wang et al., 2013). Commonly, decolorization of azo dyes occurs in the conventional systems as aerobic, facultative anaerobic and anaerobic situations by the different types of bacteria. Under anaerobic situation with the assistance of the azoreductase enzyme, degradation of the azo dyes is occurred by the reductive break in the azo groups. This phenomenon leads to the generation of colorless compounds such as aromatic amines with the high potential of danger (Saratale et al., 2011). It has been reported that the bio-altered products of some dyes like aromatic amines relative to the parental structure of dyes have revealed carcinogenic, mutagenic and toxic characteristics while parent dyes do not have direct toxicity (Balapure et al., 2014). In this similar situation, the integration of biotechnology researches by the fungi and bacteria with the chemical and physical systems have been suggested for the treatment of wastewater containing dyestuffs (Telke et al., 2009). The integration of the biological methods with AOPs methods is considered in the divers investigation. The incorporation of the methods considerably can abate the quantity of chemical materials and the price of operation (Brindha et al., 2018). Therefore, in this study, RB-5 chose as the model dye for biodecolorization and detoxification. RB-5 is a water-soluble dye with reactive functional groups that can covalently bond with the fiber. RB-5 is consumed for dying wool, cotton, and nylon (El Bouraie & El Din, 2016). A bacterial strain that isolated from vermicompost manure was used for decolorization of RB-5. The ability of the isolated bacterial strain to decolorization of different initial RB-5 concentration and several azo dyes were studied under anaerobic condition. Finally, the toxicity of RB-5 and its metabolites was investigated by phytotoxicity test.

MATERIALS AND METHODS

Reactive Black-5 ($C_{26}H_{21}N_5Na_4O_{19}S_6$; MW 991.82 g/mol; λmax 598nm, Class diazo), Methyl Orange, Methyl Red, Erichrome Black-T, and Acid Blue-113 were purchased from Sigma Aldrich. Nutrient Broth was purchased from Liofilchem (Italy). H_2O_2 was purchased from Merck.

In this investigation, the bacterial strain (gram positive rod) was isolated from the vermicompost manure (from garden waste in the of Environmental Health lab of Shahrekord University of Medical Sciences). 1 g of sieved vermicompost added to nutrient broth medium containing 100 mg/L of RB-5 for decolorization. After that, serial dilution of the colorless solution was carried out and cultured on the nutrient agar medium containing RB-5. For the isolation of the pure microorganism, the strict subculture was done. Finally, a loop of the pure colony added to nutrient broth supplemented by RB-5 for decolorization. Then for the adaptation of the bacterial strain with dye, the medium was enriched by the higher concentration of RB-5 and incubated with the previous pure colorless solution of the single bacterial strain. For bidecolorization experiments, stock solution of RB-5 was prepared in 2000 mg/L, passed from 0.22 µm sterile filter and then diluted to ideal concentration (800, 850, 950 and 1400 mg/L) by adding to nutrient broth medium. The mediums were incubated at 35 °C, pH 7 with 4% (v/v) inoculum size under anaerobic condition. Also, the ability of the bacterial strain for decolorization of 200 mg/L of other azo dyes (EBT, MO, MR, and AB-113) was studied. For analysis of decolorization at time intervals 12, 24, and 36 h, samples were taken and centrifuged at 4500 rpm for 20 min. The absorbance of clear supernatant was measured by the spectrophotometer at 598 nm (DR6000 Hach Germany). Decolorization efficiency was estimated by Eq. 1.

$$\text{Decolorization efficiency (\%)} = C_i - C_t/C_i \times 100 \quad (1)$$

where C_i and C_t are the initial and final concentration at time period of t, respectively. All of the experiments were done in triplicate and the results are shown by mean ± SD.

To assay the toxicity of treated wastewater of RB-5 (800 mg/L), phytotoxicity test was done for Triticum aestivum and Maize. In this step the germination of the seeds was investigated under watering include distilled water (as control), untreated wastewater, treated wastewater by the single bacterial strain (ethyl acetate extracted by-products) and treated wastewater (extracted by-products) in combination with UV-C (6W) + 100 mM H_2O_2 at room temperature. The seeds were irrigated by 10 ml of the solutions for 5 days. The results of phytotoxicity have been shown by germination (%). The percentage of germination was estimated by Eq. 2.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

For accessing the effect of dye concentration on the removal of color, four levels (800, 850, 950 and 1400 mg/L) were chosen. As shown in Fig. 1, at the lower concentration of RB-5 (800 mg/L), the efficiency was above 90% at 12h (91.94 ± 0.99) and for the other time intervals, 24 and 36h efficiency reached 93.29 ± 0.87 and 94.79 ± 0.68, respectively. The decolorization process was performed faster at lower concentration however, for the 850 and 950 mg/L, the decolorization started to abate after 12h. At this time the decolorization efficiency was lower than 90% (84.56 ± 1.13 and 78.69 ± 1.33, respectively). Afterward, efficiency raised to 94.06 ± 0.35 and 93.6 ± 1.26% within 36h for the concentration of 850 and 950 mg/ L, respectively. For 800, 850 and 950 mg/L of RB-5, the efficiency was high and when it boosted to 1400 mg/L, a sharp decline was seen at 12h (32.28 ± 1.07).

Even with time from 12 to 36h, only half of the concentration of 1400 mg/L was removed (51.57 ± 0.39). The effect of dye concentration on the reduction of decolorization has also been reported in various researches (Daâssi et al., 2013; Khan et al., 2014; Patel et al., 2012; Palanivelan et al., 2019). Higher concentration (1400 mg/L) might need more time because of the toxicity of dye and intermediate products on microorganisms (Tan et al., 2013). The growth of microorganisms stopped at the higher concentration of dyes (Tan et al., 2016) but in this study, the isolated microorganism (gram positive rod) could tolerate and decolorize high concentration of RB-5 in the shorter time rather than the other microorganisms. In the study of (Zhang et al., 2019) on the bio-decolorization of RB-5 (100-1500 mg/L) only for the concentrations of 100-600 mg/L, the efficiency was above 80% and the lowest efficiency was obtained for 1500 mg/L (48%) within 48h. Removal of RB-5 was 91.9% and 87% within 15 days by the immobilized and suspended fungus (Pérez-Grisales et al., 2019). Also, in the other researches the removal efficiency of RB-5 was estimated at 91% and 100% for 50 and 200 mg/L in 24h, respectively (Chen et al., 2011; Qingxiang et al., 2008). Therefore, by comparing the bacterial strain with the other microorganisms, it can be concluded that the strain has a high ability to remove dyes and can be used for acceptable decolorization of the wastewater with the high concentration of dyes.

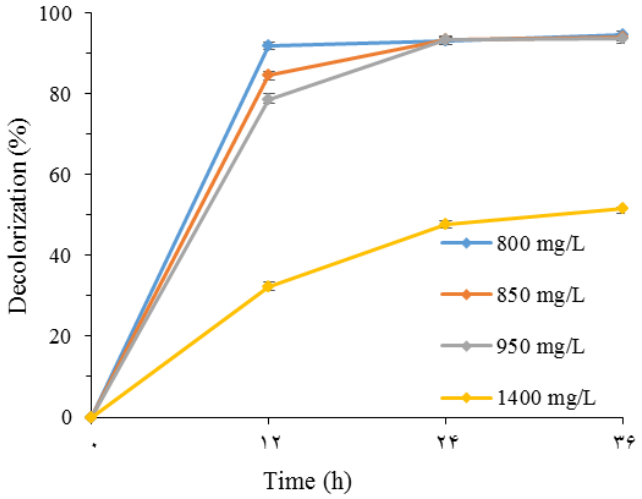
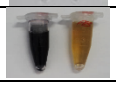


Fig. 1. effect of dye concentration on decolorization

The ability of the single bacterial strain investigated using different azo dyes to simulate real textile wastewater. As shown in Table. 2, MR and MO had the maximum efficiency and both of them were decolorized less than 12h. But EBT and AB-113 were decolorized within 24h. MO and MR have lower molecular weight and a simpler chemical structure than the EBT and AB-113. According to the reports, the removal efficiency of divers kinds of azo dyes has related to the chemical structure of dyes. Those colors that, have lower molecular weight and uncomplicated structure have been decolorized faster than the other colors by complicated structure (Cui et al., 2014). High decolorization of MR and MO in a short time was reported in different researches. Providencia rettgeri could decolorize 200 mg/L of MR more than 90% efficiency within 12h (Olukanni et al., 2019) and 250 µM of MR was decolorized in 120 min by 99% efficiency (Dong et al., 2019). Also, complete bio-decolorization of 600 mg/L and 150 mg/L of MO within 12 h was reported by (Chen et

al., 2018; Akansha et al., 2019). The results of the present study are in accordance with the research of (Wang et al., 2013). They found that for the higher molecular weight dyes (Reactive red M5B, Reactive blue-4 and Trypan blue), decolorization efficiency was lower than the dyes with low molecular weight by Bacillus sp. YZU1 (Methyl red and Methyl orange). It should be noted that the efficiency for AB-113 was lower than the other dyes, which could be due to the attendance of Sulfonate groups in the structure of dye. MR doesn't have any sulfonate group, while MO and EBT have one and also AB-113 has two sulfonate groups. (Cui et al., 2014) reported that sulfonate groups can cause persistence to decolorization process.

Table 1. decolorization of different azo dyes by the bacterial strain

Dye	Class	Chemical Formula	MW (g/mol)	λ_{max} (nm)	Decolorization (%)	
MR	mono-azo	$C_{15}H_{15}N_3O_2$	269.304	410	94.29 ± 0.38	
MO	mono-azo	$C_{14}H_{14}N_3NaO_3S$	327.33	502	92.10 ± 0.15	
EBT	mono-azo	$C_{20}H_{12}N_3O_7SNa$	461.38	526	90.83 ± 0.48	
AB-113	di-azo	$C_{32}H_{21}N_5Na_2O_6S_2$	681.649	566	88.95 ± 0.30	

Also, toxicity of untreated RB-5 and treated RB-5 were investigated in two-step. In the first step, Triticum aestivum and Maize irrigated by DW, untreated RB-5 and treated RB-5 by the bacterial stain. All the seeds germinated in DW, and also for untreated RB-5 the germination was 90 and 100% for Maize and Triticum aestivum. But for the treated RB-5, none of the seeds didn't germinate (as shown in Table. 3). The results showed that RB-5 in parent form wasn't toxic for the seeds but when it treated by the single bacterial strain, the dye broke down to the more harmful products than the parent form. Thus owing to the generation of more poisonous compounds, T.aestivum and Maize couldn't germinate. The toxicity of intermediates of RB-5 can be due to the formation and accumulation of aromatic amines during anaerobic treatment. (Brindha et al., 2018) reported that aromatic amines formed, during anaerobic degradation of azo dyes are toxic. The toxicity of metabolites from biodegradation of azo dyes has been investigated in several studies. Evaluation of toxicity of by-products of Acid blue-61 degradation by filamentous fungi A. terreus on Lactuca sativa seeds showed that due to their toxicity, they had inhibitory effects on root growth (Almeida & Corso, 2019). Production of poisonous by-products in the batch reactor during anaerobic removal of Direct black-22 was observed. The presence of poisonous aromatic amines affected the bioluminescence properties of Vibrio fischeri (50% prohibition). While untreated wastewater containing DB-22 did not has acute poisonous effects on Vibrio fischeri (Menezes et al., 2019). (Hu, 2001) investigated the toxicity of different dyes like Reactive Red-22, Direct Blue-15, Direct Violet-9, Leather dye, and Reactive Violet-2 and reported when these dyes were degraded by pseudomonas luteola, their toxicity increased rather than the parent form of dyes. In the second step, UV-C + H_2O_2 (AOPs) used as the post-treatment process. The previous solution (treated and completely decolorized by the bacterial strain) treated again with UV-C + 100 mM H_2O_2 for 90 min. After that, irrigation of seeds was carried out by this solution and the solutions in the previous stage. In this

step, both of the seeds germinated completely (as shown in Fig. 2 and Fig. 3). H_2O_2 in the existence of UV-C irradiation leads to formation of OH• and acts as a strong oxidative factor (as Eq. 3). Therefore, hydroxyl radicals converted poisonous compounds to the less poisonous compounds for seeds which cause to germination of them. It is concluded that poisonous products of anaerobic degradation of RB-5 have been destroyed during the UV-C/ H_2O_2 process. Since the textile wastewater contains carcinogenic compounds that are generated during the reductive mechanism in the aquatic environment, therefore this wastewater is the most potentially hazardous among the other industries (Khalid et al., 2013). According to the mentioned items, the combination processes can be an appropriate option in the areas that textile wastewater is consumed for the irrigation of plants and crops. Also decolorization of RB-5 carried out in combination of biological treatment by Pseudomonas aeruginosa KY284155 and UV-A, UV-C plus TiO_2 as the post-treatment system (Hashem et al., 2018) and in the other research, decolorization and detoxification of Mordant yellow-10 was performed by biological process using Pseudomonas aeruginosa BRPO3 and Photo-Fenton process (Brindha et al., 2018). Also, detoxification of Mordant yellow-10 was performed by UAPBD (up-flow anaerobic packed bed reactor) and Photo-Fenton processes and then Eisenia fetida used for toxicity study. The results showed more toxicity for the effluent of UAPBD on Eisenia fetida due to the presence of aromatic amines. But, no toxicity was observed for the effluent of the UAPBD+Photo-Fenton process (Brindha et al., 2019).

$$H_2O_2 + h\upsilon \longrightarrow 2 \bullet OH \quad (3)$$

Table 2. phytotoxicity of RB-5 and the its by-products from degradation process after treatment

Observation	T. aestivum				Maize			
	DW	UWW	TWW	TWW + UV-C / H_2O_2	DW	UWW	TWW	TWW + UV-C / H_2O_2
Germination	100%	100%	0%	100%	100%	90%	0%	100%

DW: Distilled Water, UWW: Untreated Wastewater, TWW: treated Wastewater, TWW + UV-C /H_2O_2: Treated Wastewater + UV-C/H_2O_2

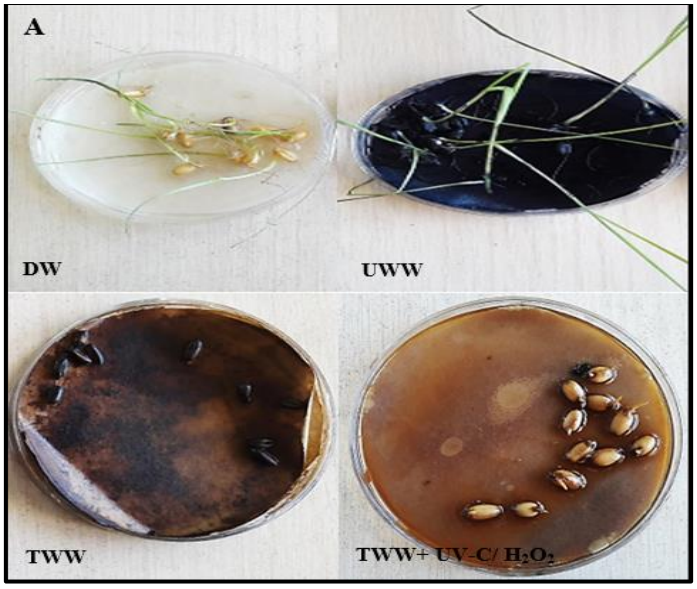


Fig. 2. Phytotoxicity study of RB-5 and its by-products. A: T.aestivum/ DW: Distilled Water, UWW: Untreated Wastewater, TWW: Treated Wastewater, TWW + UV-C/H_2O_2 : Treated Wastewater + UV-C/H_2O_2

Fig. 3. Phytotoxicity study of RB-5 and its by-products. B: Maize/ DW: Distilled Water, UWW: Untreated Wastewater, TWW: Treated Wastewater, TWW + UV-C/H_2O_2: Treated Wastewater + UV-C/H_2O_2

CONCLUSION

In this study, the bacterial strain was isolated from vermicompost manure for decolorization of RB-5 azo dye. The isolated bacterial strain had acceptable efficiency in the removal of dyes. The efficiency above 90% was observed at 35 °C and pH 7 under anaerobic condition for RB-5 and other azo dyes MR, MO, EBT and AB-113. This study demonstrated that the usage of non-native along with the native species is an effective solution in the treatment of textile wastewater. Also because of the formation of toxic compounds in the anaerobic treatment by the isolated bacterial strain, UV-C/H_2O_2 used as post treatment process to convert them to the less toxic compounds. Future work will be accomplished to identify of the isolated bacterial strain by polymerase chain reaction.

ACKNOWLEDGMENT

The authors are thankful to environmental health engineering laboratory of Shahrekord University of Medical Sciences.

GRANT SUPPORT DETAILS

The present research has been financially supported by Shahrekord University of Medical Sciences, Iran (grant No 3634).

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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