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Effect of some Organic co-pollutants on Decolorization of Reactive Violet 1 dye by an Indigenous Microbial Strain from Textile Wastewater

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Abstract

Textile wastewaters contain substantial concentration of dyes, salts, organic chemicals and solvents. The dyes not only deteriorate water quality due to color, but also adversely affect aquatic food chain and human beings due to their toxicity. Bioremediation of dyes using suitable microbes is an environment friendly approach. However, bioremediation potential is sometimes affected due to the presence of other pollutants in the wastewater. The present study reports the effect of some common solvents of textile industry (toluene, formaldehyde and acetic acid) on decolorization of toxic and recalcitrant Reactive violet 1 dye. Indigenous bacterium isolated from textile wastewater *Nesterenkonia lacusekhoensis* was used in the study. The effect of each of the solvents (1% conc.) was studied on dye degradation (100 mg/l dye concentration) under static condition by studying bacterial growth and dye decolorization. The results showed variable effects of solvents on the growth of the bacterium. While no growth was observed in the reaction mixture containing formaldehyde, the microbe was able to grow and remediate the dye in the presence of toluene and acetic acid. Bacterial growth was however, better in the presence of the dye when other solvents are absent. Decolorization was 96.8% when dye alone was used, while it was somewhat reduced in the presence of toluene (70.6%) and acetic acid (82.3%). The study shows that while the microbe has excellent capability to decolorize the RV 1 dye, it can also grow and degrade the dye quite efficiently in the presence of co-pollutants like toluene and acetic acid, which are present in textile wastewater.

Introduction

Urbanization and rapid industrialization have led to environmental pollution with detrimental effects on human health and ecosystem. Use of dyes for various industrial

processes which include paper and pulp, plastics, cloth dyeing and printing leading to considerable increase and in turn leading to the release of dye intoxicated industrial wastewaters (Aksu, 2005). Textile dyes contribute as a major source of pollution, with textile industries consuming the major share in India (Lavanya *et al*., 2014). The textile sector of India contributes up to 14% of the total industrial production in the country (Lavanya *et al*., 2014). With most of these dyes being toxic, their presence in industrial effluents is of great environmental concern, as they are recalcitrant in nature and usually resistant to degradation by microbes (Pagga and Brown, 1986). Reactive dyes are the most widely used class of dyes all over the world reaching to the amount of 178,000 tons in 2004 (Ghaly *et al*., 2014) and with a market share of 60-70% (Cinar *et al*., 2008; Sen and Demirer, 2003). Reactive azo dyes used commonly in textile industry have complex and stable structures with variety of bright colors that are quite resistant to physical agents like water, light and heat (Xie *et al*., 2008) and many of these are reported to have mutagenic and carcinogenic effects (Singh and Chadha, 2016). Around 20-50% of the reactive dyes are known to remain in aqueous phase during the process of dyeing, eventually leading to production of colored water (Gebrati *et al*., 2011). Reactive dye molecules being highly water soluble are generally known to pass through conventional method of activated sludge or municipal wastewater treatment systems, remaining virtually unchanged (Willmot *et al*., 1998). The product quality is demand from the textile manufacturing and consumers account for high wash stability and for a varied range of colors, demanding the need of reactive dyes even if they come with non-biodegradable properties (Cooper, 1998).

Along with the dye usage other auxiliary chemicals are used during the maturation process, finally leading to the production of complex wastewater varying in characteristics (Sen, 2003; Spagni *et al*., 2012). There are about more than 8,000 chemicals associated with the process of dyeing (Kant, 2012). These chemicals range from inorganic compounds and other elements to organic compounds, used for desizing, scouring, dyeing, bleaching, printing and finishing (Santos *et al*., 2007). The generated wastewater is also characterized by high pH, temperature and organic load and extreme color (Mountassir *et al*., 2003). These pollutants with carcinogenic and mutagenic properties (Sandra *et al*., 2000) show recalcitrance towards biodegradation and also posses inhibiting potential on microbes. Under some cases dye can breakdown under anaerobic condition to form potential carcinogenic components that can show bioaccumulation in the food chain (Banat *et al*., 1996). The possible toxicity and carcinogenicity posed by the textile dyes has been a major cause of concern (Khan and Hussain, 2007). Furthermore, highly colored wastewaters block sunlight penetration and oxygen which is essential for aquatic organisms (Crini, 2006).

Toxic nature of Reactive dyes

Reactive dyes are commonly incorporated for dyeing cotton, as they come with high wet-fastness property (Trotman, 1984). Reactive dyes are the prominent and widely

used class of dye embedded with various reactive groups. These dyes add to increase in COD, BOD and pH change of the wastewater and receiving water bodies (Gowri *et al*., 2014). These dyes have complex structure with organic rings and double bonds representing the color. They form covalent bond with the hydroxyl groups of the cellulosic fibres (Gohl and Vilensky, 1983). The monochlorotriazinyl dyes, also called azo dyes react with vinyl sulphonyl and chloride groups forming a bond with cellulose fibre (Trotman, 1984). Reactive dyes are harmful and their toxicity has been confirmed on various organisms. The genetoxicity (Dogan *et al*., 2005), mutagenecity (Mathur *et al*., 2005) and carcinogenicity (Roos *et al*., 2005) tests have been conducted. Effect of textile dyes has been studied on frog embryo by performing teratogenesis assay (Birhanli, 2005). Mutagenecity tests have also been studied with human keratinocytes for reactive dyes (Wollin and Gorlitz, 2004). Ames test has also been applied for testing the adverse effects of these dyes (Mathur *et al*., 2005). The most commonly appearing hazard of reactive dyes is respiratory problem due to inhalation of dye, which can affect the immune system. Symptoms like watery eyes, itching, sneezing and wheezing has also been observed (Ozkurt *et al*., 2012). An adverse effect of the dyes has also been found on the aquatic systems (Richardson, 1983). Thus, industrial effluents must be treated to remove reactive dyes before their discharge into the environment (Wong *et al*., 2003).

Along with the dyes certain organic solvents are also used in textile industry, which act as additives to enhance the quality of the color and the fabric. Formaldehyde used to enhance dye adsorption on the fibers and also used in the finishing process in textile industry as an anti-wrinkle agent is one of the key contaminants in the textile and dyeing wastewater (Sarayu and Sandhya., 2012). Formaldehyde is reported to cause allergic contact dermatitis and is a known toxic solvent (Akarslan and Demiralay, 2015). Toluene is another organic solvent, which is found in the wastewaters, which is used as a cleaning solvent throughout the printing process and is carcinogenic in nature (Joshi *et al*., 2003). US Environmental Protection Agency has classified toluene as a primary pollutant with adverse effect on human health, with carcinogenic properties (Fu *et al*., 2016).Acetic acid, which is used as a neutralizing agent in textile industry (Madhav *et al*., 2018) is also reported in wastewaters. These organic compounds, though present in small amounts act as co-pollutants in the dye-laden wastewaters of textile and dyeing industry. Many of the dyes are aromatic hydrocarbon derivatives of toluene, benzene, naphthalene and phenol (Singh and Chadha, 2016) and thus have high toxicity.

Exploiting the bioremediating potential of many bacteria, cyanobacteria, fungi and microalgae has been in focus in the recent past (Kaushik *et al*., 2011; Bayoumi *et al*., 2014, Moosvi *et al*., 2005) with extensive investigations on various operational parameters. However, several organic co-pollutants that may be present in the wastewater and which are likely to influence the bioremediation capacity of the microbes have not been paid much attention. The present study was therefore, carried out to with the aim to investigate the potential of *Nesterenkonialacusekhoensis,* isolated from textile effluent to

degrade reactive violet dye in the presence of some organic solvents used in textile industry, to explore its potential applicability in bioremediation of textile wastewater.

Materials and Methods

Bacterial inoculum: *Nesterenkonia lacusekhoensis*was the bacterial strainused in the present study which had been previously isolated in our laboratory from textile effluent and has been reported to be alkaliphilic in nature (Bhattacharya *et al*., 2017). The bacterial cells were cultivated in Nutrient Broth (NB) medium (pH 11.5), by spiking a loopful of the bacterium in 50ml medium and incubated overnight at 35 °C in an incubator shaker at 200 rpm.

Experimental set-up: Reactive Violet 1 (RV 1) dye was purchased from local textile market of Ludhiana, Punjab and it showed lambda max at 550 nm. RV 1 is an azo dye with complex aromatic ring structure as shown in Figure 1. Stock solution (10,000 mg/l) of the dye was prepared, which was further diluted with distilled water to 100 mg/l concentration. All organic chemicals used were of analytical grade.

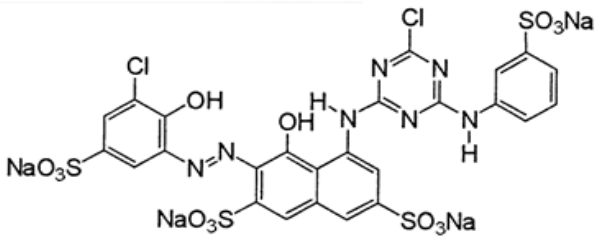

Figure 1 Structure of Reactive violet 1 dye.

Treatments used in the study included (i) Reactive violet 1 dye (100 mg/l) in 50 ml of Nutrient Brothwith 7.5% bacterial inoculum, serving as control,(ii) Reaction mixture as in (i) +1 % toluene, (iii) Reaction mixture as in (i) +1% formaldehyde and (iv) Reaction mixture as in (i) +1 % acetic acid. All experiments were conducted in triplicates.

The bacterium was grown in small sterilized flasks with 50ml NB medium (initially inoculated with 7.5% inoculum) andincubated at 35 °C for 72 h. Growth was determined using UV-vis spectrophotometer at 600 nm, which was done by reading absorbance of the bacterial cells in NB and again, after centrifuging the medium, reading the OD of the supernatant. By subtracting the absorbance of the centrifuged supernatant from that of the original NB sample containing bacterial mass represented bacterial growth.The organic solvents (1%) were individually added in 100 mg/l of dye, in

presence of nutrient broth media (50 ml, pH 11.5). The remediation of textile dye in presence of toxic solvents was studied at its lambda max (550 nm) using UV-vis spectrophotometer. Dye decolorization was calculated using the formula:

$$\text{Decolorization (\%)} = (A_i - A_f) \times 100/A_i$$

where A_i and A_f represent initial and final absorbance of dye solution after treatment.

Spectral scans of RV 1 dye was done using UV-vis spectrophotometer at intervals to know the changes in various peaks with time during decolorization by the bacterium, to gain insight into the process of dye decolorization.

Results and Discussion

Bacterial growth response: Bacterial cell growth in the presence of reactive violet dye and that along with the three organic solvents is depicted in Table 1. The absorbance of the bacterial cells in NB medium were determined by subtracting the absorbance attained by reading at 600 nm of 1 ml sample (without centrifugation) and the reading attained after centrifugation of the same sample extracted, after every 24 h of incubation.

Table 1. Growth of *Nesterenkonialacusekhoensis* EMLA3 cells in Nutrient broth medium in the presence of Reactive Violet 1 dye (100 mg/l) and organic solvents (1% each).

Bacterial growth (O.D. at 600 nm)					
Incubaton time (h)	*Control	Reactive Violet Dye	Toluene + dye	Formaldehyde +dye	Acetic acid+dye
0	0.3962	0.4728	0.3735	0.3405	0.3313
24	0.8472	0.9425	1.0639	0.3330	1.3482
48	1.1727	1.2297	1.4322	0.3346	1.3621
72	1.4139	1.4238	1.6865	0.3374	1.3967

*Control (Bacterium in NB without dye)

For the process of degradation to occur, it is very important that the microbe should be able to survive and grow in the medium containing toxic compounds. Though toluene and acetic acid are known to inhibit bacterial growth (Nahar *et al*., 2000; Ryssel *et al*., 2009), the present strain showed continuous increase in cell absorbance in the presence of the dye alone and also in combination with toluene and acetic acid. However, in the presence of formaldehyde, the absorbance was less than that in control or that in

dye alone. This indicated inability of the bacterium to tolerate formaldehyde. This could be attributed to high toxicity of formaldehyde to microbial cells (Chen *et al*., 2016). The bacterial growth shows the potential of the microbe to survive and grow in the presence of the reactive dye and co-contaminants like toluene and acetic acid at high alkaline pH.

Dye decolorization: Decolorization of the dye by the microbe in static conditions and no residual color on the microbial pellet after the treatment indicate that the microbe removes the dye by degrading it and not by biosorption. The results of spectral scan of the dye during 72h of treatment also clearly suggest that the dye is degraded by the bacterium to simple compounds. While the microbe decolorized about 78% of the dye in 24h, it increased to more than 96% in 48h (Figure 2). The peak at 325 nm (Figure 2) corresponds to naphthyl rings of Reactive violet 1 dye. During the dye decolorization process the pi-bond of the conjugated chromophore in the dye was broken, also observed in the degradation of Reactive violet 5 R (Jain *et al*., 2012). With the cleavage of the bond intermediates corresponding to naphthyl ring breakdown would have accumulated in the UV region. The increase in absorbance at with increase in time at 24, 48 and 72 h in the UV region confirms the formation of new products. While in the visible region, the absorbance at the lambda max (550 nm) was found to show decrease in absorbance with increase in time indicating degradation of dye.

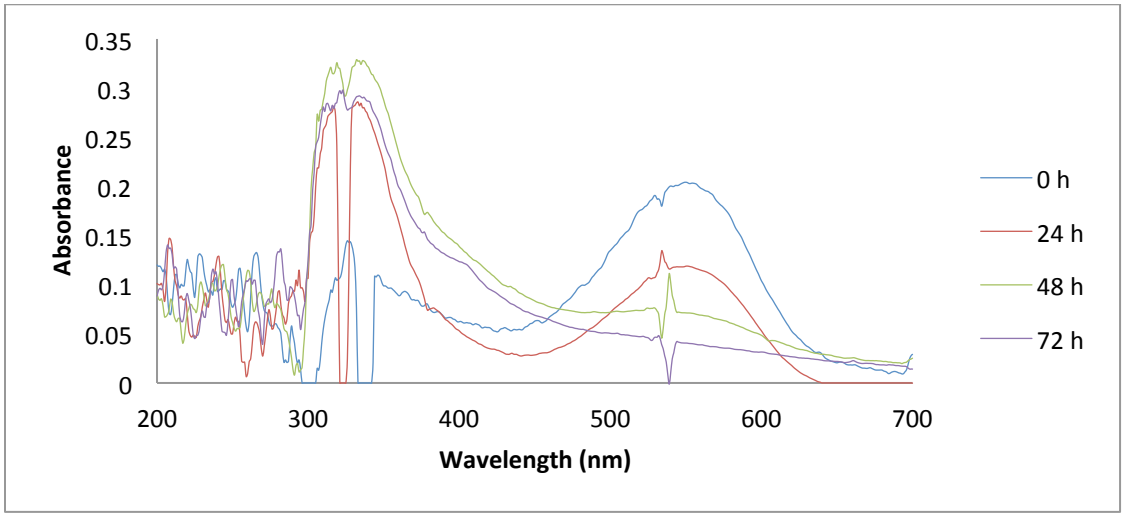

Figure 2 UV-vis spectral scanning of Reactive violet 1 dye during decolorization by the bacterium.

Decolorization of the reactive violet dye by *Nesterenkonia* as influenced by the three common organic solvents may be seen in Table 2. In the presence of toluene and acetic acid, the decolorization process was relatively slow initially, showing only 4% and 14% removal, respectively, in the first 24h. The decolorization process improved in next 24h showing up to 71% and 82% dye removal (Table 2). On the other hand, no decolorization of the dye was observed in the presence of formaldehyde, which seems to

be due to toxic effect of the organic solvent. This is in consonance with our earlier observations on poor tolerance and growth of the microbe in the presence of formaldehyde.

The results clearly indicate that *Nesterenkonia* is tolerant to toluene and acetic acid in low concentration and in the presence of these solvents; it can successfully decolorize the reactive violet dye. The positive results for dye remediation in the presence of toluene and acetic acid suggest that the toxicity of these solvents might have been be reduced or avoided by the microbe by some suitable mechanism. The dyes are reported to induce the activity of some degrading enzymes bound in the inner part of the cytoplasm membrane. The hydrophobic ability of the compound for accumulation in cytoplasmic membrane is decreased by hydroxylation of compound. Usually the degradation pathway begins with incorporation of hydroxyl group into pollutant structure (Cao *et al*., 2013). Therefore, increasing the polarity leads to its higher water solubility and increased availability to microbial attack. The microbe seems to not only mineralize the dye, but is also likely to breakdown the two organic solvents.

Table 2. RV Dye decolorization* (%) in presence of organic solvents by alkaliphilic bacterium.

Dye decolorization (%)				
Incubation time (h)	Dye only	Dye+Toluene	Dye+Formaldehyde	Dye+Acetic acid
0	0.0	0.0	0.0	0.0
24	77.6	4.0	0.0	14.0
48	96.1	57.4	0.0	70.7
72	96.8	70.6	0.0	82.3

Control (without bacterium) showed no dye decolorization in 72h.

Conclusion

The study clearly shows high efficacy of the microbe to successfully grow in the presence of RV 1 dye and decolorize it by degrading the complex aromatic structure. It is also able to tolerate and decolorize the reactive violet dye in the presence of toluene and acetic acid, which are known toxic solvents, thus, advocating its potential application for dye containing textile wastewaters where these organic solvents are present as co-pollutants. Though a time lag is observed in growth as well as decolorization in the presence of toluene and acetic acid as co-pollutants, the final removal is quite

appreciable. However, the microbe cannot be used to remediate the dye in the presence of formaldehyde, which seems to be too toxic for its growth.

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