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*Full Length Research Paper*

# Biotreatment of anthraquinone dye Drimarene Blue $K_2RL$

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**Drimarene Blue (Db) $K_2RL$ is a reactive anthraquinone dye, used extensively in textile industry, due to poor adsorbability to textile fiber; it has a higher exhaustion rate in wastewater. The dye is toxic, carcinogenic, mutagenic and resistant to degradation. Decolorization of this dye was studied in two different systems. Shake flask study, using the same immobilized fungus *Aspergillus niger* SA1 with increasing concentration (10 - 300 ppm) of dye resulted in 75% decolorization in 24 h with 10 ppm concentration, while the higher the concentration of dye, the lower the values (68% at 25 ppm, 40% at 50 ppm, 11% at 100 ppm, 3% at 200 ppm and 2% at 300 ppm) of decolorization ($p < 0.05$). Simulated textile effluent containing 10 ppm dye Db $K_2RL$ was tested aerobically using sludge in stirred tank reactor (STR) at 30ºC for 8 days. The values achieved of chemical oxygen demand (COD) reduction and decolorization were 84% ($r = 0.968$) and 30% ($r = 0.905$), respectively. This study revealed that immobilized fungus *A. niger* SA1 have potential, while sludge containing bacterial consortium have limited potential to be used as bioremediation for textile dye Drimarene Blue $K_2RL$.**

**Key words:** Immobilized fungus, Drimarene Blue $K_2RL$, stirred tank reactor, COD.

## INTRODUCTION

Reactive Anthraquinone dyes represent the second largest class of textile dyes, after azo dyes and are used extensively in the textile industry due to their wide array of color shades, ease of application and minimal energy consumption (Aspland, 1997). Anthraquinone dyes are resistant to degradation due to their fused aromatic structure, which remain colored for a long time (Banat et al., 1996). Additionally, most of these dyes are toxic, carcinogenic and mutagenic (Itoh et al., 1996). Decolorization of anthraquinone dyes has received much attention due to recalcitrant nature (Laszlo, 1995). Reactive dyes are not readily removed by typical wastewater treatment processes due to their inherent properties, such as stability and resistance towards light or oxidizing agents (Lee et al., 2005).

Dye removal from wastewater with traditional physiochemical methods, such as coagulation, adsorption and oxidation with ozone is expensive, can generate large volumes of sludge and usually require the addition of environmental hazardous chemical additives (Robinson et al., 2001). Considering drawbacks in aforementioned treatments, microbial remediation techniques have gained much attention in the last few decades. Microbial decolorization and degradation is an environment friendly and cost-competitive substitute to different conventional treatment technologies (Verma and Madamwar, 2003; Gogate and Pandit, 2004).

Dyes are removed by fungi by biosorption (Fu and Viraraghavan, 2000), biodegradation (Conneely et al., 1999) and enzymatic mineralization (Lignin peroxidase, Manganese peroxidase, Manganese independent peroxidase and laccases (Wesenberg et al., 2003; Pointing and Vrijmoed, 2000). Immobilization of living microorganisms has been described as valuable in biological wastewater treatment (Katzbauer et al., 1995). Application of Immobilized living biomass of fungal strains have been proved more practical than the cell-free system, specifically when they expected to show adsorption as well as enzymatic capabilities of dyes degradation (Lin et al., 2003; Rojek et al., 2004).

Sludge treatment of wastes is often an effective and

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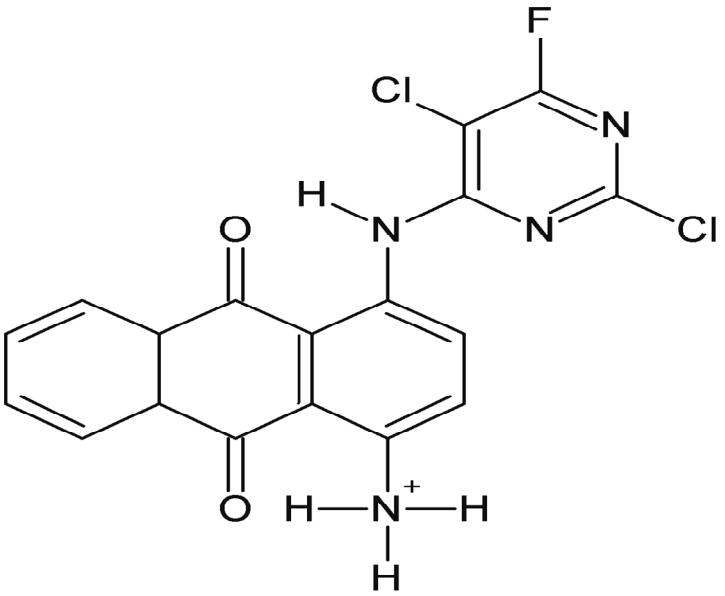

**Figure 1.** Structure of dye Drimarene Blue $K_2RL$ (Siddiqui et al., 2009).

highly economic system for reducing organic pollutants in wastewater. A fair amount of research has been conducted assessing the viability of using activated sludge to treat textile effluents (Pagga and Brown, 1986). Aerobic microbes cannot reduce azo linkages; their ability to destroy dye chromogens is less than anaerobic bacterium. However, aerobic sludges have been successfully used to stabilize dye metabolites (Brown and Laboureur, 1983).

Several types of bioreactors have been developed for treatment of dyes (Hao et al., 2000; Melo and Oliveira, 2001) and selecting an appropriate reactor is essential in improving the economy and efficiency of process (Chen et al., 2003). There is need of bioreactor system that can sustain production of high level of enzymes for long period together with controlled growth microorganisms (Chang et al., 2001; Zhang et al., 1999).

A significant amount of research has already been done on the decolorization/degradation of azo dyes and their related products (Perey et al., 2002), however, limited information exists in case of reactive anthraxquinone dyes (Epolito, 2004). Therefore, there is need to explore effective and efficient treatment systems of microbial stains for the mineralization of anthraquinone dyes.

In present study, a reactive Anthraquinone dye Drimarene Blue $K_2RL$ is used, which is known for its excessively usage in textile industry. Drimarene Blue $K_2RL$ and related dyes, due to their poor adsorbability to textile fiber has a higher exhaustion rate in wastewater. Fungal strain *Aspergillus niger* SA1, previously isolated from dyes wastewater, was immobilized on support material Scotch-Brite™ (80% polyester and 20% nylon) and were applied in shake flasks, while sludge containing bacterial consortium in stirred tank to explore their biodecolorization/biodegradation abilities for Db $K_2RL$ in Simulated Textile Effluent.

## MATERIALS AND METHODS

### Chemicals

The majority of chemical compounds and media components were procured from BDH laboratory chemical division (Pool Dorset, England) and Buch Sigma chemicals Co; St, Lois, E-Merck (Darmstadt, Germany). The investigated commercial dye Drimarene Blue (Db) $K_2RL$ (Figure 1) (Anthraquinone based dye) was obtained from Kohinoor Textile Mill, Rawalpindi, Pakistan.

### Saboraud dextrose broth

Saboraud dextrose broth (Merck), pH 5, was used for cultivation of fungal strain.

### Composition of simulated textile effluent

Simulated textile effluent (STE) was made by adding per liter of distilled water; Acetic Acid (99.9%) 0.15 ml, $(NH_2)_2CO$ 108.0 mg, $KH_2PO_4$ 67.0 mg, $NaHCO_3$ 840.0 mg, $MgSO_4$. $7H_2O$ 38.0 mg, $CaCl_2$ 21.0 mg, $FeCl_3$ .$6H_2O$ 7.0 mg, glucose 860 mg (Luangdilok and Panswad, 2000) and Db $K_2RL$ 10 mg, pH (5) of effluent was adjusted by using 0.1 M HCl and NaOH.

### Fungal strain used

Fungal strain, *A. niger* SA1, was collected from Microbiology Research Lab (MRL), Quaid-i-Azam University, Islamabad,

Pakistan. This strain was refreshed on Sabouraud dextrose agar medium at pH 5. This fungal strain was previously isolated from Kohinoor Textile Mill, Rawalpindi, Pakistan and were identified (Ali et al., 2007) on the basis of morphological characters.



**Figure 2a.** Immobilized *Aspergillus niger* SA1 on Scotch brite (Siddiqui et al., 2009).

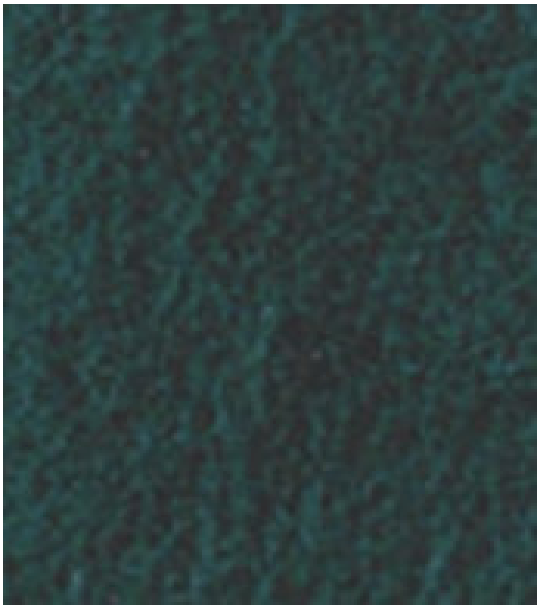

**Figure 2b.** Scotch brite without Immobilized fungus (Siddiqui et al., 2009).

**Immobilization support material**

Scotch-Brite™ (Spain) a kitchen scouring pad (80% polyester and 20% nylon, green color, size: 3 × 3 cm, thickness: 0.8 mm) was used as immobilization support material.

**Inoculum preparation (Spores suspension)**

Fungal strain *A. niger* SA1 were grown on Sabouraud dextrose agar (pH 5) plate for a week at 28ºC. The fungal spores were scratched and picked up with a loop from mature colony of fungal strain. The loop containing fungal spores was then dipped and mixed 15 times in 100 ml autoclaved distilled water containing 0.05% Tween 80 solution. After vigorous shaking, 1 ml of the inoculum was poured on the hemocytometer and was observed under microscope. The observation showed average $7.35 \times 10^3$ spores per ml of inoculum of the strain. This inoculum was used for further experimentation. The whole process was carried out in aseptic conditions in laminar flow hood. The inoculum was stored in refrigerator at 4ºC.

**Immobilization of fungal strain on support material**

Scotch-Brite™ (Spain) was used as immobilization support material. Small pieces (size: 3 × 3 cm, thickness: 0.8 mm) of Scotch-Brite™ (Spain) were used as immobilization support material (Rodriguez et al., 2004). These pieces were thoroughly washed with distilled water and sterilized in autoclave prior to use (Linko, 1991). Flask having 150 ml Sabouraud Dextrose broth (pH 5) and 15 pieces of Scotch-Brite™ (Spain) were added to it. It was inoculated with 10 ml spores suspension of *A. niger* SA1 and was placed in rotary shaker incubator (INNOVA TM 4330, New Brunswick Scientific) at 30ºC, 120 rpm for 1 week. The immobilized Scotch brite and without immobilization are shown in Figure 2.

**Sample of sludge**

Sample of sludge was collected from aeration tank, in sterilized reagent bottle (Pyrex) having 1 l capacity from sewage wastewater treatment plant of Capital Development Authority, I- 9/4, Islamabad, Pakistan. Sample of sludge was kept at 4ºC in Microbiology Research Laboratory (MRL), Islamabad.

**Treatment of Db $K_2$RL using immobilized fungus in shake flask with increasing concentration**

Simulated textile effluent (STE) containing different concentrations (10, 25, 50, 100, 200 and 300 ppm) (triplicate) of dye Drimarene Blue (Db) $K_2$RL were used to test the decolorizing ability of the same immobilized fungus in shake flasks. For this purpose under optimized conditions, keeping pH 3 of the effluent, experiment was performed at 30ºC on shaker incubator (INNOVA TM 4330, New Brunswick Scientific) for 6 days. After every 24 h, the same immobilized fungus was transferred to new flask having increased concentration of dye.

A control experiment was also run, in which pieces of Scotch-Brite™ (Spain) without immobilized fungus were studied for their ability to adsorb (abiotic loss) the dye Drimarene Blue $K_2$RL in STE by overnight incubation in shake flask (Rodriguez et al., 2004). The apparent dye removal by the fungal strain was critically examined into/onto the hyphae by microscope.

**Configuration and operation of bioreactor**

Stirred tank reactor (STR) was designed (using tank of 5 L made of PP (Kartel, Italy) with height of 14 Inches and diameter of 7 inches. System was operated at room temperature 20 - 25ºC and pH was

kept 3. Sludge was used as inoculum (10%) in Stirred Tank Reactor. Inoculum was thoroughly mixed before use. A 1 l of simulated textile effluent containing (10 mg $l^{-1}$) of drimarene blue $K_2$RL with pH 3 (triplicate) was used for study. The contents of Reactor were agitated with stirrer (ES, VELP Scientifica, made in Europe) at 200 rpm and were aerated continuously. The reactor was run continuously for 8 days. A control experiment without inoculum was also run in parallel.

**Analytical methods**

***Spectrophotometric analysis***

Samples collected (2 ml) from different experiments were centrifuged (Beckman Coulter TM, Germany) at 12000 rpm for 10 min. The supernatants collected from centrifuged samples was read at 620 nm (λ max of Drimarene Blue $K_2$RL) using spectrophotometer (Agilent spectrophotometer). The dye free Simulated Textile Effluent was used as a blank. Standard curves of known concentrations of dye were made for measuring its concentration in the samples. Percent removal of dye in Simulated Textile Effluent was determined .

***Chemical oxygen demand (COD)***

Chemical oxygen demand (COD) of treated samples were analyzed by closed reflux colorimetric method (APHA, 5220 D). COD was estimated taking absorbance at 600 nm. COD was measured as COD mg $O_2$ /l.

***Statistical analysis***

Data obtained during experiments was statistically analyzed using SPSS software. Probability (p-value) less than 0.05 and 0.01 was considered significant and highly significant, respectively. The results were expressed in terms of Means and Standard error (SE±).

***Precaution***

Experimental work was carried out under standard sterilize conditions. Each experiment was conducted in triplicate in order to avoid errors.

## RESULTS

This study clearly validated the role of an indigenous brown-rot fungal isolate *A. niger* SA1 and sludge containing bacterial consortium for achieving decolorizetion/degradation of reactive anthraquinone dye Drimarene Blue $K_2$RL. Application of Immobilized fungus revealed biosorption/bioadsorption to be the predominant dye removal phenomenon.

### Treatment of simulated textile effluent containing dye Db $K_2$RL with immobilized fungus in shake flasks

In shake flask experiment the same immobilized fungus was gradually exposed to increasing concentration of Db $K_2$RL (10 - 300 ppm) with 24 h retention time for each concentration. Effluent having 10 ppm Db $K_2$RL showed 75% (S.E ±1.3) decolorization ability, 25 ppm showed 68% (S.E ±1.2) decolorization, 50 ppm showed up to 40% (S.E ±1.5), 100 ppm showed 11% (S.E ±1.8), 200 ppm showed 3% (S.E ±2.1) and 300 ppm showed up to 2% (S.E ±1.9) decolorization ability (significant at $p < 0.05$). The results showed that with increasing concentration of Db $K_2$RL using same immobilized fungus, the decolorization ability decreased ($r = 0.854$) (Figure 3). The result showed 1% loss of dye due to abiotic activity of Scotch-Brite™ (Spain).

### Stirred tank reactor (STR) for treatment of STE containing Db $K_2$RL

Simulated textile effluent containing 10 ppm of Db $K_2$RL was prepared with pH 3 (COD = 650 mg/l). The simulated textile effluent was treated with 10% sludge in stirred tank reactor. The reactor was run continuously for 8 days. After every 24 h, samples were taken for spectrophotometer and COD analysis. Considerable values of decolorization and reduction in COD were achieved. The higher values achieved of % COD reduction and decolorization were 84% (S.E. ±2.1) and 30% (S.E. ±1.1), respectively (Figure 4). Time course of treatment of effluent revealed that decolorization was increased ($r = 0.968$) considerably between 96 - 120 h, but no further change in decolorization was observed. While percent COD reduction was increased ($r = 0.905$) between 96 - 120 h, then no further decrease in COD was observed. Results of COD reduction and decolorization were quite improved to permissible limits.

## DISCUSSION

Current research work has appreciably validated the role of a fungus *A. niger* SA1 and sludge in the removal of an important reactive anthraquinone dye from a simulated textile effluent. For this purpose immobilized fungus *A. niger* SA1 and sludge were tested in shake flasks and sludge in bioreactor system in decolorization/degradation experiments.

Apparently, dye removal in the present study was merely seen due to biosorption/bioadsorption of fungal hyphae. Likewise, few other studies have also clearly mentioned biosorption/bioadsorption of certain brown rot fungi (*A. niger* and *Aspergillus foetidus*) (Ali et al., 2007; Fu and Viraraghavan, 2000; Knapp and Newby, 1995; Sumathi and Manju, 2000) as the primary dyes removal phenomenon coupled with electrostatic pull between the positively charged cell wall and negatively charge dyes (Aksu et al., 1999; Aksu and Tezer, 2000). Dyes removal by *A. niger* SA1 was microscopically found more due to

biosorption/bioadsorption into/onto fungal hyphae as was reported by Fu and Viraraghavan (2000).

Concentration of dye have vital role in decolorization



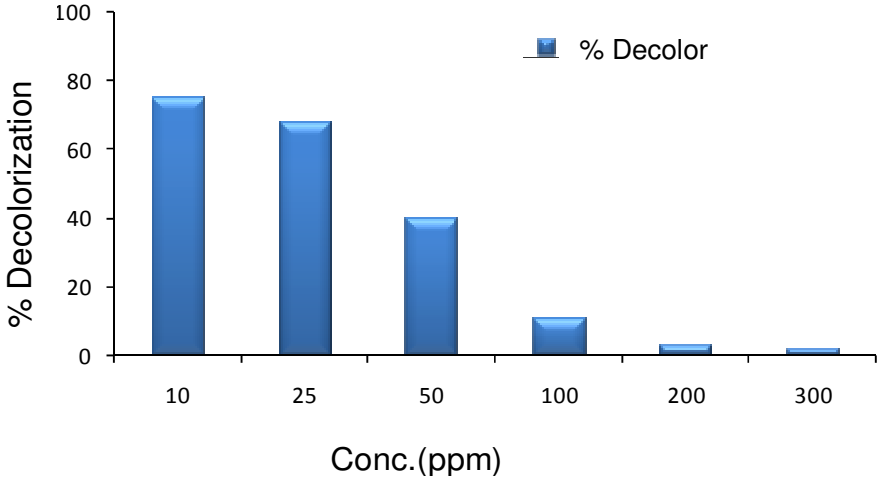


**Figure 3.** Decolorization of Db $K_2$RL dye in shake flask using immobilized *Aspergillus niger* SA1 with retention time of 24 h.

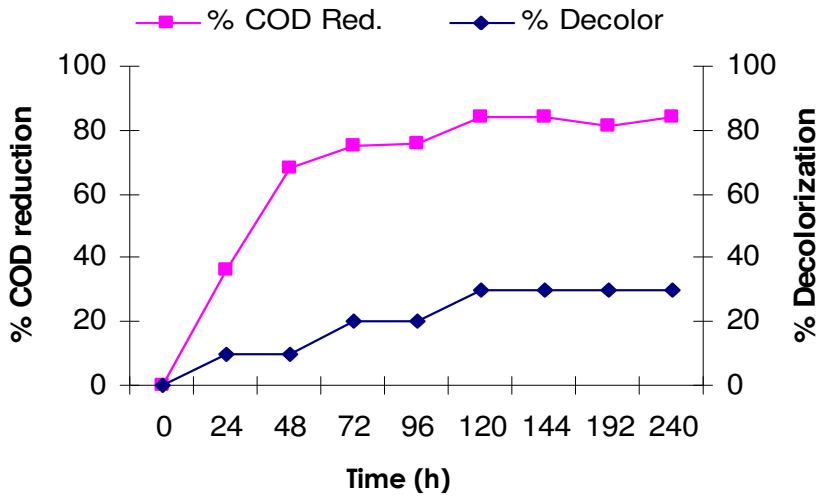


**Figure 4.** Stirred tank reactor for treatment of Db $K_2$RL with concentration of 10 ppm.

process, different concentrations of dye have been applied. We have examined the decolorization of Db $K_2$RL with increasing concentration (10 - 300 ppm). Decolorization ability of immobilized fungus decreased with increasing concentration of dye Drimarene Blue $K_2$RL, but the immobilized fungus was still able to decolorize (2%) the dye even at higher concentration 300 ppm. This happens due to the high dye concentration, which may negatively affect the color removal efficiently, either by exceeding the reactors biological dye capacity or by causing toxicity to the biomass (Isik and Sponza, 2005). However increase in concentration (> 100 $mgl^{-1}$) of dye (Reactive blue) at times proved to be toxic, thereby limiting the decolorization activity of *Aspergillus* spp. (Ramya et al., 2007). Albanis et al. (2000) reported that the removal of dyes decreases with increasing concentration of dye from 10 - 60 $mgl^{-1}$ showing that the process was highly dependent on the concentration of dye solution.

The higher values achieved of COD and decolorization were 84 and 30%, respectively. The color removal efficiency is poor in stirred tank reactor and it could be due to biosorption alone. A fair amount of research has been conducted assessing the viability of using sludge to treat textile effluents (Zissi et al., 1997). Only few studies have described the successful usage of aerobic sludge for color removal. The successful removal of color was reported in a study by aerobic sludge system with color reduction of 75 and 85% respectively (Wallace, 2001). Primary mechanism for removal of dyes on sludge

systems may occur by adsorption onto the cell wall of microbes (Pagga and Taeger, 1994). Biodegradation of dyes can be accomplished when catabolic activities, present in mixed microbial communities, complement 050 Afr. J. Environ. Sci. Technol.

each other (Knackmuss, 1996). With different types of activated sludge treatment methods, the following removal are normally achieved: about 90% of BOD, 40 - 50% of COD and 10 - 30% of color (Mittal and Gupta, 1996).

## Conclusion

The study revealed that immobilized fungal *Aspegillus niger* SA1 have potential and sludge containing bacterial consortium have limited potential to be used as bioremediation of dye Drimarene Blue $K_2$RL. Further studies will be performed to validate on whether the removal is due to biotransformation or biosorption and additional information regarding the possibility of microbial contamination might be needed.


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