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Full Length Research Paper

Up-flow immobilized fungal Column Reactor for the Treatment of Anthraquinone dye Drimarene blue K_2RL

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This research work is on the decolorization of a reactive anthraquinone dye Drimarene blue (Db) K_2RL, which is known for its markedly usage in textile industry. Due to poor adsorbability to textile fiber, it has a higher exhaustion rate in wastewater. The main objective of our research work was to evaluate the potential of an Up-flow Column reactor (UFCR) (13x1.7//) containing the fungal strain, *Aspergillus niger* SA1 (immobilized on support material Scotch-Brite™) for the decolorization of a dye, Db K_2RL, in simulated textile effluent. Different concentrations of dye in the effluent were treated in the reactor for 24 h, with a flow rate of 10 mL^{-1} at hydraulic retention time (HRT) of 10 h. Using anoxic UFCR, decolorization of the effluent was observed maximum, that is, 94.26% at 10 ppm of dye; however, it reduced to 58.51% at 300 ppm of dye. A trend towards increase (≤ 15%) in decolorization of effluent was noted, when the effluent was aerated prior to treatment. Recycling of the effluent containing dye increased the decolorization (85% at 130 ppm of dye; 66% at 500 ppm of dye), however, further recycling decreased the rate of decolorization, which might be due to desorption by the immobilized fungus. The results of these findings providing important insights into the development of effective treatment technology for bioremediation of textile dyes.

Key words: Drimarene blue K_2RL, *Aspergillus niger* SA1, immobilized, up-flow column reactor.

INTRODUCTION

Synthetic dyes are widely used in textile dyeing, paper printing, color photography and food processing (Liu et al., 2004; Pearce et al., 2003). Wastewater containing synthetic dyes can be accountable to multiple environmental problems, in part due to its recalcitrant and xenobiotic nature. Effluents from the textile industries containing dye are highly coloured and are therefore visually identifiable (O'Neil et al., 1999; Kilic et al., 2007).

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).

Up till now scientists have been trying to develop a single and economical method for the treatment of dyes in the textile waste water but still it remains a big challenge (Santos et al., 2007). 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 through biosorption (Fu and

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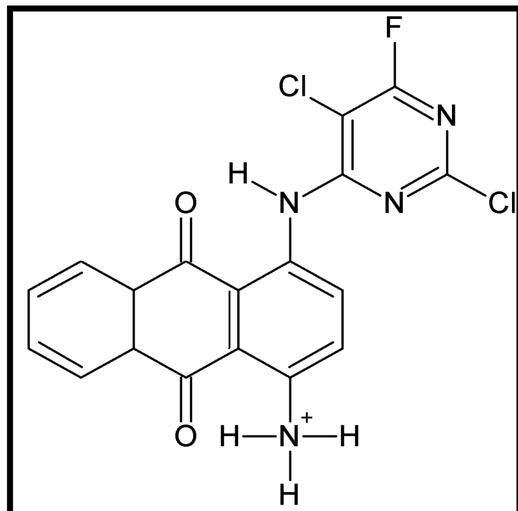

Figure 1. Structure of dye Drimarene blue K2RL.

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 (Aksu and Tezer, 2000; Coulibaly et al., 2003; Lin et al., 2003; Rojek et al., 2004).

Several types of bioreactors have been developed for treatment of dyes; however, most efficient decolorization achieved when fungal mycelium was immobilized in the reactor (Hao et al., 2000; Melo and Oliveira, 2001). Waste-rwater treatment has attracted increasing interest using immobilized cell bioreactors (Chen et al., 2003). Immobilized microbial systems greatly improved bioreactor efficiency for dyes decolorization; for instance, increasing process stability and tolerance to shock loadings, allowing higher treatment capacity per unit biomass, generating relatively less biological sludge, repeated and long term applications (Chen et al., 2003; Chang et al., 2001). Selecting an appropriate reactor is essential in improving the economy and efficiency of immobilized cell 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 of fungi. The widely used systems were stirred tank reactor, air lift, bubble column, fixed bed bioreactor, rotating disk reactor and silicon membrane reactor (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 anthraquinone 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 (Db) K_2RL (Figure 1) was used, which is known for its markedly 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 were immobilized on scotch brite (80% polyester and 20% nylon) and were applied in an Up-flow Column Reactor to explore their biodecolorization abilities for Db K_2RL dye 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) was used for immobilization of fungal strains. It was made by adding per litre of distilled water; dextrose 40 g and peptone 10 g. pH (5) of broth medium was adjusted by using 0.1 M HCl and NaOH. Agar (15 g l^{-1}) was used as solidifying agent in the media when required in the experiments.

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, *Aspergillus 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.

Immobilization support material

Scotch-Brite^{TM} (Spain) was used as immobilization support material (80% polyester and 20% nylon, color green).

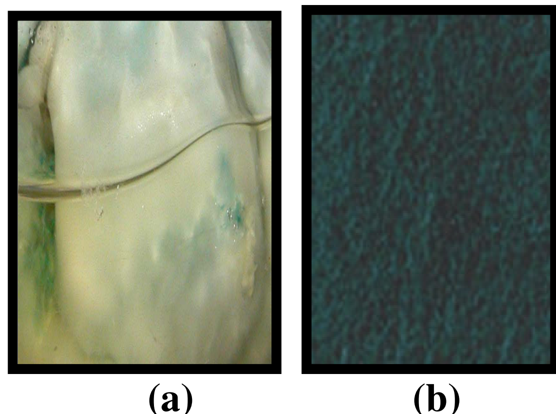

Figure 2. (a) Immobilized *A. niger* SA1 on Scotch brite. (b) Scotch brite without Immobilized fungus.

Inoculum preparation for bioreactor (Spores suspension)

Fungal strains 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 soultion. After vigorous shaking, 1 ml of the inoculum was poured on the hemocytometer and was observed under microscope. The observation showed average 7.35×10^3 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^{TM} (Spain) was used as immobilization support material. Small pieces (size: 3 x 3 cm, thickness: 0.8 mm) of Scotch-Brite^{TM} (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^{TM} (Spain) were added to it. It was inoculated with 10 ml spores suspension of *Aspergillus 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.

Configurations and operation of bioreactor

Column reactor (UFCR) for treatment of simulated textile effluent containing drimarene blue K_2RL

The Up-flow immobilized fungal column reactor (Modified from Manjinder et al., 2006) was built from borosilicate glass column (13 x 1.7//) at glass blowing shop, Peshawar University, Pakistan. The glass column was filled with immobilized pieces (Scotch Brite) of *Aspergillus niger* SA1 (45 Scotch Brite pieces) to a bed height of 7 inch. Column was connected at lower side inlet to a tubing (Silicon, Sigma Aldrich) attached to feed tank and outlet was connected to a tubing that was attached to a sample collection tank (sedimentation tank). The UFCR was fed in from feed tank containing STE in Up-flow mode by a peristaltic pump (EYELA-microtube pump MP3, Tokyo) (Figure. 3) at an average flow rate of 10 mlh^{-1} with an average hydraulic retention time (HRT) of 10 h. Simulated textile effluent containing Drimarene blue (Db) K_2RL with increasing concentration (10, 25, 50,100,200,300 ppm) with pH 5 was treated through Up-flow immobilized Column Reactor (UFCR). For each concentration (240 ml each), column was run for 24 h. A wash of 100 ml citrate buffer was given before and after each treatment. The column was run continuously for 6 days.

Aerobic column reactor was designed with slight modification from Manjinder et al. (2006) and Sharma et al. (2004). Here filtered air was pumped with an air compressor (Rocker-300) into the feed tank containing dye Drimarene blue (Db) K_2RL. The rest of treatment was same as that of Anoxic reactor.

Effect of recycling on decolorization of Drimarene blue K_2RL in Up-flow immobilized column reactor was tested for 2 different concentrations, that is, 130, 500 ppm. Sample (480 ml effluent) having 130 ppm Db K_2RL was treated for 48 h and then it was recycled 6 times up to 288 h. In another experiment using the same procedure decolorization ability was tested for higher concentration of Db K_2RL (500 ppm) (480 ml) with recycling effect.

A control column reactor was also run, in which pieces of Scotch-Brite^{TM} (Spain) were checked for their ability to adsorb the dye by overnight incubation in Column reactor having STE containing dye Drimarene blue K_2RL to check the abiotic loss of dye (Rodriguez et al., 2004). The apparent dye removal by the fungal strain was critically examined into/onto the hyphae by microscope.

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_2RL) 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 by using the following formula:

$$\% \text{ Decolorisation} = \frac{\text{Initial concentration of dye} - \text{Final Concentration of dye}}{\text{Initial Concentration of Dye}} \times 100$$

Precaution

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

RESULTS

The present study clearly validated the role of an indigenous brown-rot fungal isolate *A. niger* SA1 for achieving enhanced decolorizationn of reactive anthraquinone dye Drimarene blue K_2RL. Application of Immobilized column reactor revealed biosorption/bioadsorption to be the predominant dyes removal phenomenon.

The immobilized fungus in anoxic Up-flow column reactor was gradually exposed to increasing concentration (10-300 ppm) of Db K_2RL (240 ml effluent for each conc. used) in simulated textile effluent. Using anoxic

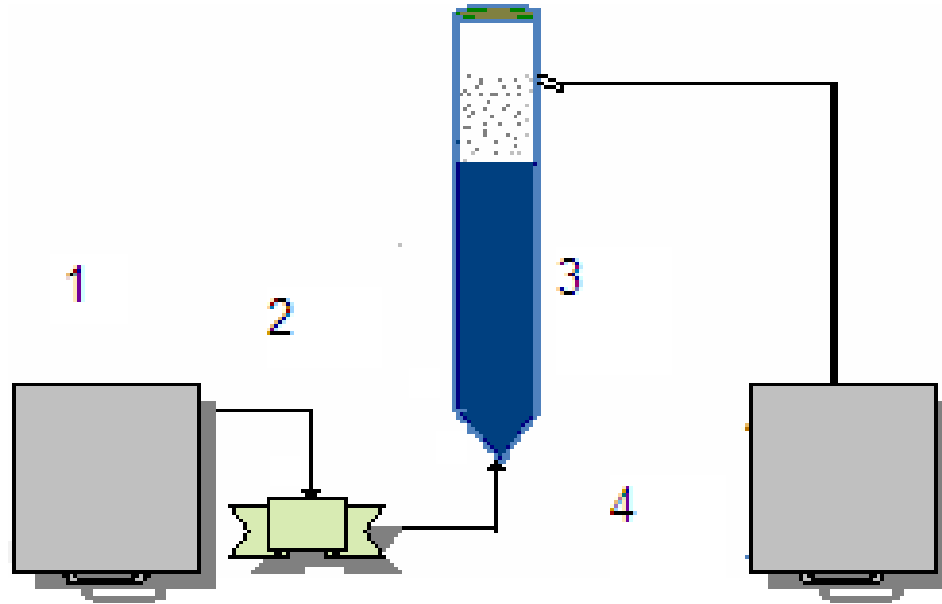

Figure 3. Flow diagram of Anoxic Up-flow column reactor. 1. Feed Tank, 2.Peristaltic Pump, 3. Up-flow column reactor, 4. Sample collection tank (Sedimentation Tank)

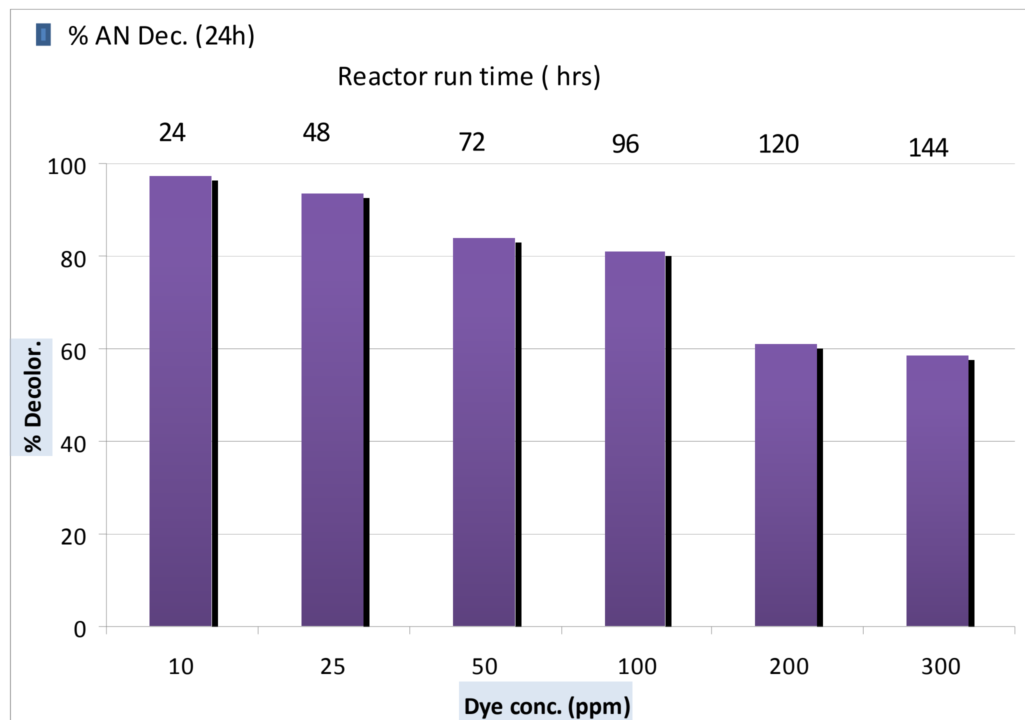

Figure 4. Decolorization of Db K_2RL dye with increasing concentration in anoxic UFCR.

UFCR, decolorization of the effluent was observed maximum i.e., 94.26% at 10 ppm of dye; however, it reduced to 58.51% at 300 of dye (Figure 4). Effect of aeration supply was investigated, which showed considerable increase in decolorization rate, that is, 99.02% at 10 ppm of dye; however, it reduced to 43.9% at 300 ppm of dye (Figure 5) as compared with anoxic UFCR. A trend towards increase (≤ 15%) in decolorization of effluent was

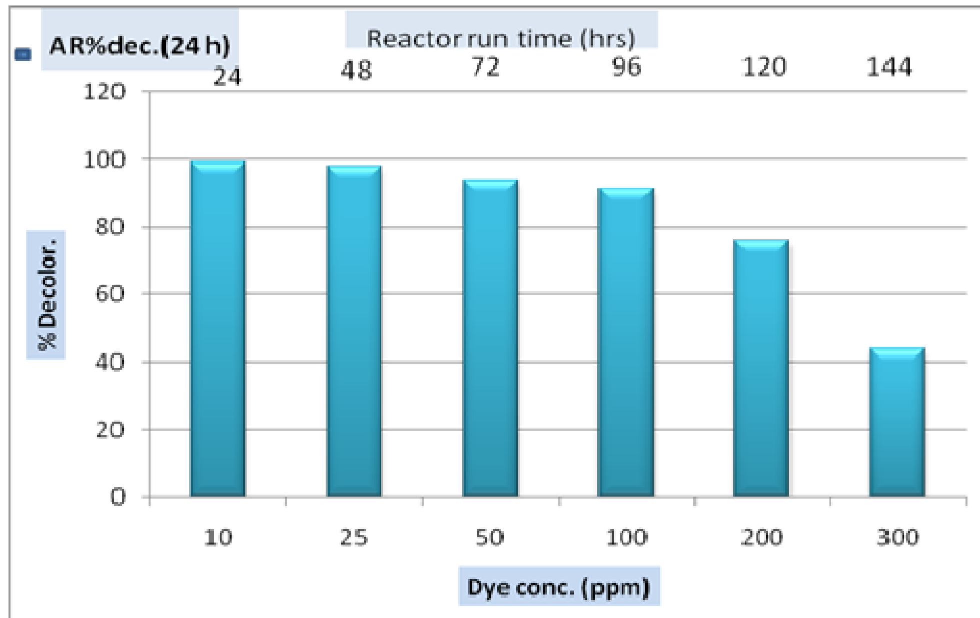

Figure 5. Decolorization of Db K_2RL dye with increasing concentration in aerobic UFCR.

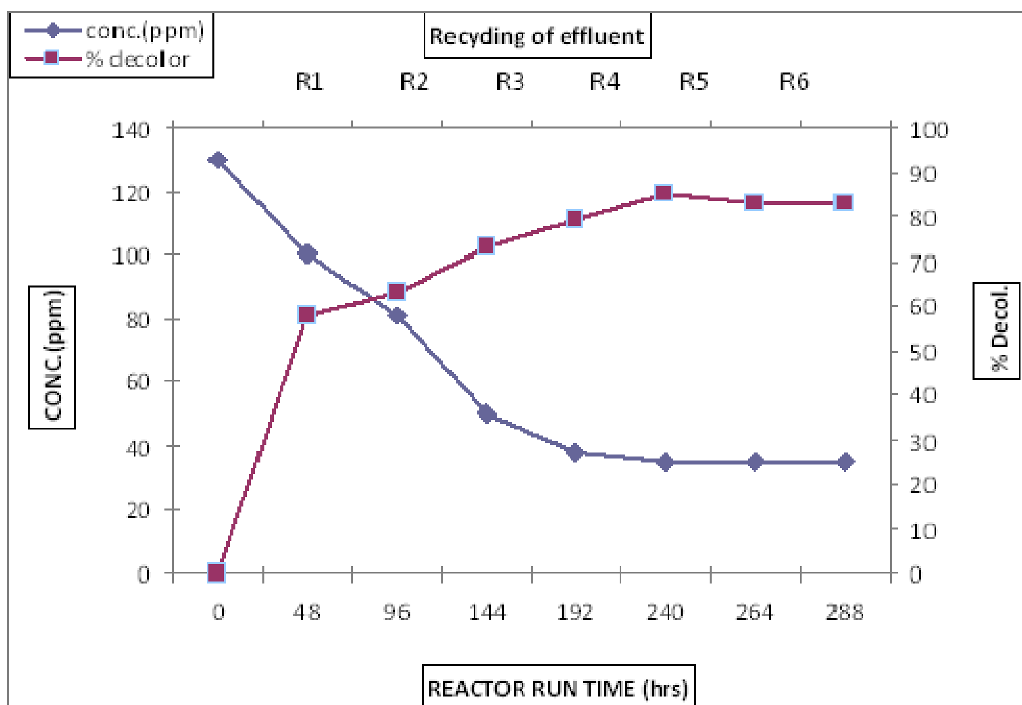

Figure 6. Effect of recycling on decolorization of Db K_2RL dye with concentration of 130 ppm (Flow Rate 10 ml/h).

noted, when the effluent was aerated prior to treatment.

Effect of recycling on decolorization of dye was evaluated for the increased rate of decolorization. 2 experiments were performed in which different concentrations (130,500 ppm) of dye were tested using recycling effect on decolorization. In the first run (cycle), 63% decolorization at 130 ppm of dye (Figure 6); 42% decolorization at 500 ppm of dye (Figure 7) was achieved. While recycling

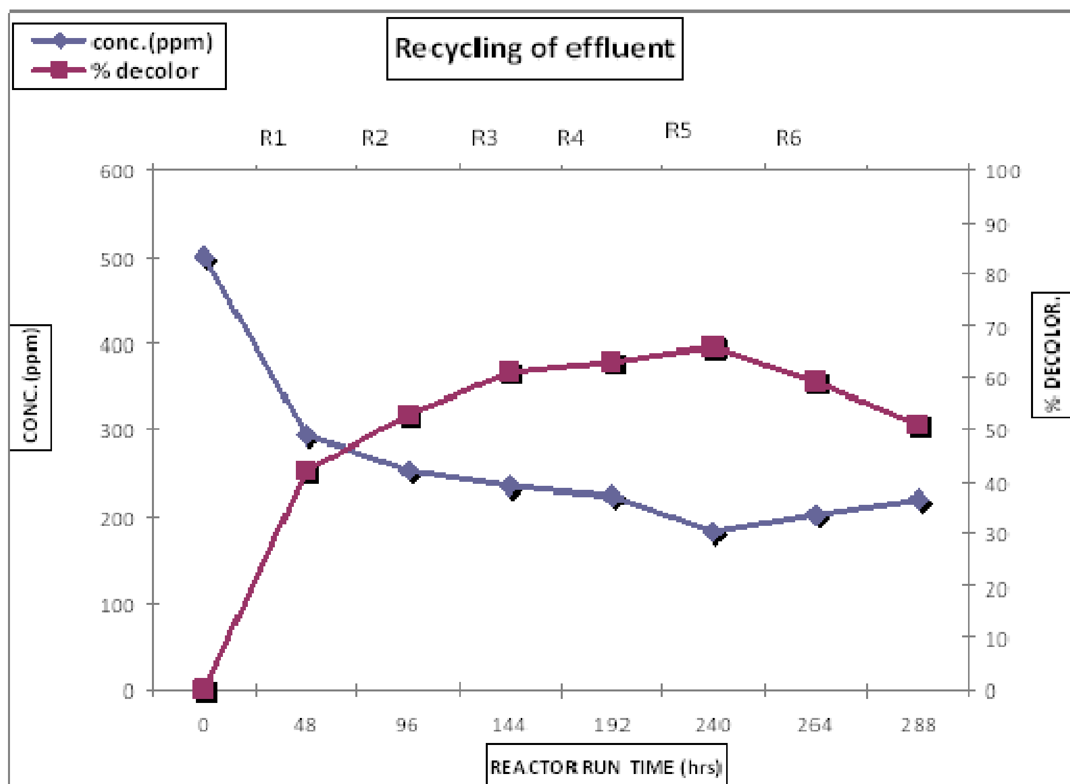

Figure 7. Effect of recycling on decolorization of Db K_2RL dye with Concentration of 500 ppm (Flow rate 10 ml/h).

of the effluent containing dye, increased the decolorization rate (85% at 130 ppm of dye; 66% at 500 ppm of dye); however, further recycling decreased the rate of decolorization by the immobilized fungus.

DISCUSSION

Current research work has appreciably validated the role of a fungal strain *A. niger* SA1 in the removal of an important reactive anthraquinone dye from a simulated textile effluent. Previously various bioreactors have been used in this context; however, each reactor showed some limitations related to its efficiency in the removal of structurally different dyes.

Apparently, dyes 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 *A. 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).

Dye concentration play a major role in decolorization process, different concentrations of dyes have been applied in bioreactors. We have examined the decolorization of Db K_2RL with increasing concentration (10-300 ppm) in anoxic Up-flow column reactor and aerobic Up-flow column reactor. Decolorization ability of immobilized fungus decreased with increasing concentration of dye Drimarene blue K_2RL, but the immobilized fungus was still able to decolorize (43.9%) the dye even at higher concentration 300 ppm. In several cases, the applied dye concentrations largely exceed the 10 - 25 mg/l range of normal concentrations in dyehouse effluents (O'Neill, 1999). 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).

The aeration supply had a crucial effect in color removal. Aeration supply in bioreactors (packed bed fungal column reactor) cause a large increase in decolorization percentages compared with those obtained in anoxic conditions (Moreira et al., 1997). The effect of aeration supply was investigated in an experiment. With the supply of aeration considerable results obtained as

compared to anoxic column reactor. A trend towards increase (≤ 15%) in decolorization of effluent was noted, when the effluent was aerated prior to treatment. As previously described in batch or continous system (Dosoretz et al., 1990; Moreira et al., 1997), aeration supply supposedly increases MnP activity, which would explain the greater decolorization efficiency attained.

Recycling of the effluent containing dye, increased decolorization (85% at 130 ppm of dye; 65% at 500 ppm of dye), however, further recycling decreased the rate of decolorization. It might be due to desorption of the dye from fungal cells especially at higher dye concentrations or long contact time or may be due to higher molecular mass, structural complexity and the presence of inhibitory groups, NH_3 in the dye (Ali and Muhammad, 2008). Further study in this direction will be highly valuable to know the details of desorption of dye from fungal cells in case of higher dye concentrations and long contact time.

Conclusion

Using anoxic UFCR, decolorization of the dye was observed maximum; however, higher the concentration of dye lowers the values of percent decolorization. A trend towards increase (≤ 15%) in decolorization of effluent was noted, when the effluent was aerated prior to treatment. Recycling of the effluent containing dye increased decolorization; however, further recycling decreased the rate of decolorization, which might be due to desorption by the immobilized fungus. 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. The results of these findings provide important insights into the development of effective treatment technology for bioremediation of textile dyes.

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