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Full Length Research Paper

Decolorization of anthroquinone based dye Vat Red 10 by *Pseudomonas desmolyticum* NCIM 2112 and *Galactomyces geotrichum* MTCC 1360

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Wastewater, from the textile and other dyestuff industries containing synthetic dyes, require prior treatment to prevent groundwater contamination. The microbial decolorization and degradation of these dyes play a pivotal role in this aspect. *Pseudomonas desmolyticum* NCIM 2112 and *Galactomyces geotrichum* MTCC 1360 could bring about oxidative degradation resulting in decolorization of this water insoluble Vat Red 10 (Novatic red 3B) dye at pH 9, at 25°C. The decolorization was measured as the decrease in absorbance maxima at 530 nm. The end product of degradation and decolorization was 2, 6-Di isopropyl Naphthalene (2, 6-DIPN), which is an important plant growth factor.

Key words: Vat dyes, vat red 10(Novatic Red3B), dye decolorization, textile, *Pseudomonas desmolyticum, Galactomyces geotrichum,* wastewater, 2, 6-DIPN.

INTRODUCTION

The discharge of large amount of toxic waste in the environment is a consequence of rapid industrialization and urbanization. One of such pollutants are the textile dyes which are very chemically stable as these have to be fast to prevent easy loss of color during washing and fading on exposure to sunlight. Large numbers of synthetic and chemically different dyes are used for various industrial applications and significant proportion appears in the form of wastewater which ultimately finds their way in the environment. The textile industry is one such industry which discharges a large proportion of these pollutants. Their presence in an environment like a water body, leads to reduction in sunlight penetrations resulting in decrease in photosynthetic activity and thus reduced dissolved oxygen content in water bodies. Depending on the class of the dyes, their loss in waste waters can range from 2% of the original concentration for basic dyes to as high as 50% for reactive dyes (O'Neill et al., 1999; Tan et al., 2000; Boer et al., 2004). Amongst these, azo and vat dyes represent the largest and most versatile class of synthetic dye (Keharia et al., 2004). Around 10,000 different dyes with annual production of more than $7x10^5$ metric tones worldwide are commercially available for various applications (McMullan et al., 2001).

Dyes on the basis of their chromophore group are classified as azo, anthraquinone, nitro, nitroso, triphenylmethane, xanthene, acridine, thiazole, sulfur, indigoid, dyes etc. Dye concentrations that are used for processing vats are typically around 1,000 mg^{-1} (Ince and Tezcanli, 1999). In order to prevent such toxic effects which has an adverse effect on the natural biodiversity, it is essential to free the environment of these coloring materials. Many physical and chemical methods including adsorption, coagulation, precipitation, filtration, and oxidation have been used for the treatment of dye-contaminated effluents. These methods, however, may generate a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage. Moreover, their municipal treatment costs are high. Various wood-rotting fungi were able to decolorize azo dyes using peroxidases or laccases. Therefore, it may be economical to develop alternative means of dye

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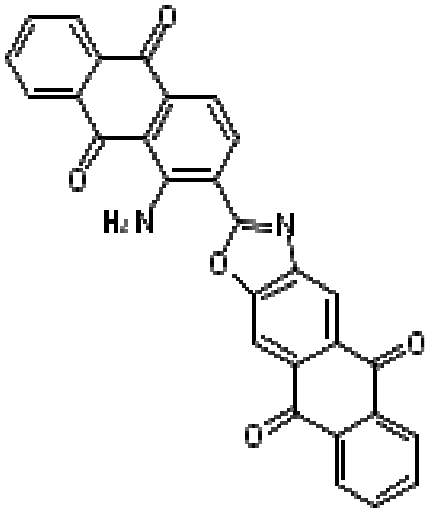

Figure 1. Chemical structure of Vat Red 10.

decolorization, such as bioremediation due to its reputetion as an environmentally friendly acceptable treatment technology. The sequential anaerobic treatment followed by aerobic bacterial degradation system has proved to be efficient in the degradation of these dyes.

Microbial decolorization and degradation is an environmental friendly and cost effective process (Verma and Madamwar, 2003). Several reports revealed the existence of a wide variety of microorganisms capable of decolorizing a wide range of dyes (Banat et al., 1996). The effectiveness of microbial treatment depends on the survival, adaptability and activity of the selected organisms (Paszecazynki et al., 1992). Anthraquinone dyes like Vat Red 10 (Novatic Red 3B), which are also classified as oxazole derivatives, are resistant to degradation due to their fused aromatic structure, which remain colored for a long time (Banat et al., 1996). The molecular structure of this dye is as given in Figure 1. It has a molecular weight of 473 Da (Venkatraman, 1971). These dyes are carcinogenic and mutagenic (Itoh et al. 1996) to humans. Decolorization of anthraquinone dyes has received much attention due to their recalcitrant nature (Laszlo, 1995).

This study investigates microbial decolorization of Vat Red 10 dye by *Pseudomonas desmolyticum* NCIM 2112 and *Galactomyces geotrichum* MTCC 1360 at high pH because these dyes are soluble in water only at alkaline pH. These can be reduced, in the presence of a reducing agent in an alkaline medium; forming a water-soluble leuko-compound. The most commonly used reducing agent in vat dyeing is sodium dithionite. However, one of the major drawbacks of this industrial dyeing process is the high amount of sulfate, sulfite, and thiosulfate in the wastewater and of the toxic sulfide formed subsequently leading to bio-corrosion of the wastewater pipelines.

MATERIALS AND METHODS

Microorganisms and culture medium

Pure cultures of *P. desmolyticum* NCIM 2112 and *G. geotrichum* MTCC 1360 were maintained on solid mineral base medium having the following composition ($NaNO_3$ 0.3%, K_2HPO_4 0.1%, $MgSO_4$ 0.05%, KCl 0.05%, yeast extract 0.02% and agar 2.5%) with 1% glucose. The culture was adapted to grow at pH 9 in the same medium at 25°C. The media used in this study were liquid mineral base medium having the same composition as aforestated:

$$\text{Decolorization rate } \% = \frac{A-B}{A} \times 100$$

A - Initial absorbance; B - Observed absorbance.

Dyes and chemicals

The commonly used vat dye for cotton dyeing- Vat Red 10 or Novatic Red 3B was used in this experiment. The other chemicals used were of analytical grade and highest purity.

Decolorization experiment

The flasks containing medium were sterilized by autoclaving. One millilitre of microbial suspension containing $120x10^6$ cells was inoculated into 100 ml of the aforementioned liquid medium with glucose and containing Vat Red 10 dye at 0.01%. The incubation was carried out on a rotary shaker 120 rpm at 25°C for 23 days. At every 7 days interval, the flasks were checked for reduction in color by comparing with control set of experiments where no bacterial culture was added. The decolorization was measured as the decrease in absorbance maxima at 530 nm.

Statistical analysis

Results obtained were the mean of three or more determinants. Analysis of the variants was carried out on all data at $P< 0.05$ using Graph Pad software (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Vat Red 10 is a vat dye which is soluble only at alkaline pH of 9. Therefore, to bring about microbiological decolorization, the organisms like *G. geotrichum* MTCC 1360 and *P. desmolyticum* NCIM 2112 were first adapted to grow at pH 9, as both these organisms were only reported to grow at pH 7. The adaptation studies were carried out in a stepwise manner grown in presence of 1% glucose. It was observed that when the adapted strain of *G. geotrichum* were grown in the presence of the dye and 1% glucose, 45% of the dye was decolorized in 23 days, of which 35% decolorization was in the first 7 days, as shown in Figure 2. It can be seen from the figure that the rate of decolorization was high during the initial period and it declined later.

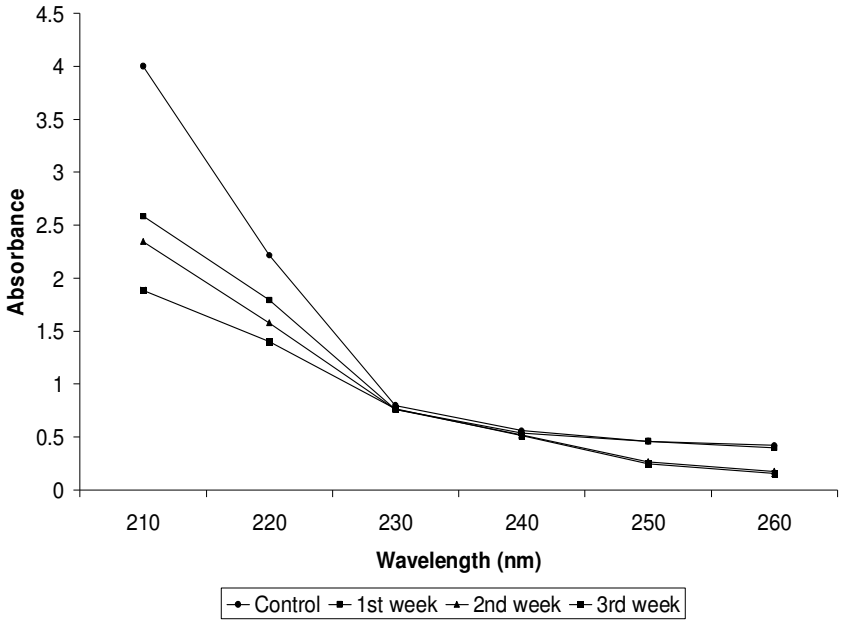

Figure 2. Decolorization of anthroquinone dye Vat Red 10 by *Galactomyces geotrichum* grown in minimal liquid base media containing 0.01% glucose. Observations were taken at regular time intervals that is after 7 days at 530 nm on UV-vis spectroscope.

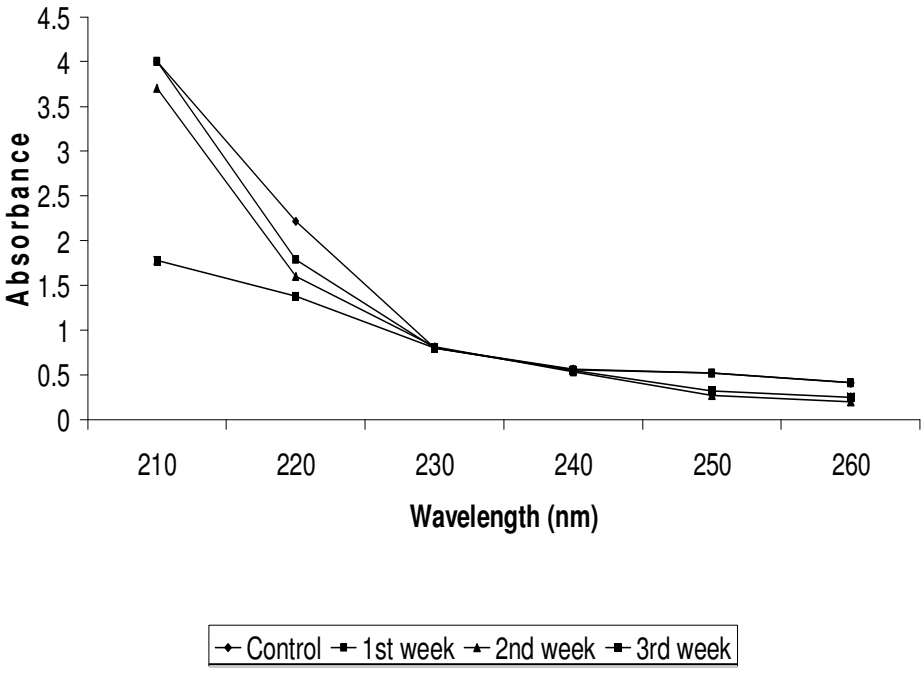

Figure 3. Decolorization of anthroquinone dye Vat Red 10 by *Pseudomonas desmolyticum* grown in minimal liquid base media containing 0.01% glucose Observations were taken place at regular time intervals that is after 7 days.

Similarly, *P. desmolyticum* NCIM 2112, in presence of glucose, could bring about 55.5% decolorization in 23 days, as shown in Figure 3. The decolorization process was aerobic as the experiments were conducted in shake flask conditions. The proposed mechanism (Figure 4) of decolorization were as per the intermediates like

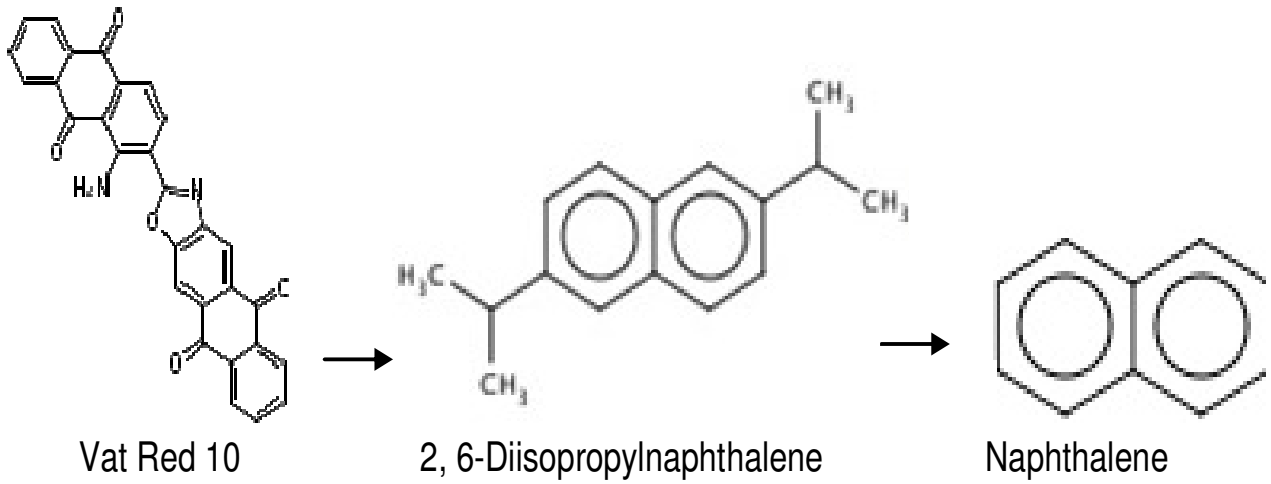

Figure 4. Proposed pathway for the decolorization of Vat Red 10 by *Galactomyces geotrichum*.

diisopropylnaphthalene and naphthalene, which were identified by GCMS as per for "diisopropylnaphthalene" (Brzozowski et al., 2007) "Naphthalene" (www.epa.gov/region01/eco/airtox/fs/naphthalene.html 2007). The dye could have been detoxified either by bioaccumulation or by biodegradation (Knapp and Newby, 1995; Sani and Banerjee, 1999). The results clearly pointed out to the fact that biodegradation was the sole means of decolorization and thus detoxification. If there was bioaccumulation, then the cells of *P. desmolyticum* and *G. geotrichum* should have shown the presence of the dye either on their outside or in the cytoplasm. This was not observed from the absorption maxima studies. Similar results were observed with *Aeromonas hydrophila* DN322 and the concerned dyes were crystal violet, malachite green, reactive red etc. (Suizhou et al., 2006).

However, crystal violet was detoxified by *Aeromonas* spp B5 by adsorption on its surface (Nobuki et al., 2000). *Aspergillus niger SA1* could detoxify anthraquinone dye – Drimarene Blue by biodegradation (Muhammad et al., 2010), *Coriolus versicolor* could degrade and detoxify the anthraquinone dye Pigment violet 12 (Itoh et al., 1998), *Trametes versicolor* could degrade 2 carpet anthraquinone dyes (Ramsay and Goode, 2004). This being a first report of its kind of microbial detoxification of such a recalcitrant like Vat Red 10 which is an anthraquinone – oxazole dye, by these 2 organisms belonging to genera *Pseudomonas* and *Galactomyces*. The end product of the degradation being a useful compound – Di-isopropyl naphthalene (2, 6-DIPN), which is a well known plant growth regulator, totally nontoxic to the environment. It is even hypothesized that this will further undergo demethylation to yield naphthalene. Therefore, biodegradation of Vat red 10 as per this investigation not only helps to protect the environment but also produces substances which are of agronomic importance.

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