

Decolorization of Textile Dye Vat Blue 66 by *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus megaterium* NCIM 2087

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Abstract: The main objective of this investigation is to detoxify the water insoluble dyes like Vat Blue 66 which is other very difficult to decolorize. Wastewater from the textile and other dyestuff industries contain significant amount of synthetic dyes that require prior treatment to prevent groundwater contamination upon being discharged. The microbial decolorization and degradation of dyes has been of considerable interest biological treatment of the wastewater containing dyes. *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus megaterium* NCIM 2087 was able to decolorize indole based dye Vat Blue 66 at pH 9, at 25°C. The addition of glucose and yeast extract in the medium enhanced decolorization process. In this investigation, it has been found that the decolorization of this dye was 70% on an average by oxidative degradation by *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus megaterium* NCIM 2087. The decolorization was measured as the decrease in absorbance maxima at 530 nm.

Key words: *Bacillus megaterium*, dye decolorization, *Pseudomonas desmolyticum*, textile, Vat dyes, Vat Blue 66, wastewater

INTRODUCTION

The treatment of textile effluents is of interest due to their toxic impacts on receiving waters. While much research has been performed to develop effective treatment technologies for wastewaters containing vat dyes, no single solution has been satisfactory for remediating the broad diversity of textile wastes. Human and ecological health concerns have prompted the government to require textile effluent discharges to have increasingly lower color and nitrogen levels. Indole dyes like Vat Blue 66, are resistant to degradation due to their fused aromatic structure, which remain colored for a long time. Several reports revealed the existence of a wide variety of microorganisms capable of decolorizing a wide range of dyes (Banat *et al.*, 1996). Around 10,000 different dyes with annual production of more than 7×10^5 metric tones worldwide are commercially available for various applications (McMullan *et al.*, 2001).

Removing dyes from textile wastewater is a difficult and expensive process. It is often not necessary to remove all the dye molecules in the treatment processes. However, it is required to decolorize the dye completely, and to transform it into such a chemical form which would be non-toxic (Perkowski *et al.*, 2003). The molecular structure of this dye is as given in Fig. 1. It has Molecular weight of 279.31 Dalton with a molecular formula: $C_{16}H_9NO_2S$ (Bruning *et al.*, 2010).

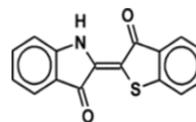


Fig. 1: Structure of vat blue 66

This study investigates microbial decolorization of Vat Blue 66 dye by *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus megaterium* NCIM 2087 at high pH because these dyes are soluble in water only at alkaline pH.

MATERIALS AND METHODS

The study was carried out during June 2010 to March 2011.

Microorganisms and culture medium: Pure cultures of *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus megaterium* NCIM 2087 were maintained on solid mineral base medium having the following composition (NaNO₃ 0.3%, K₂HPO₄ 0.1% MgSO₄ 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 above.

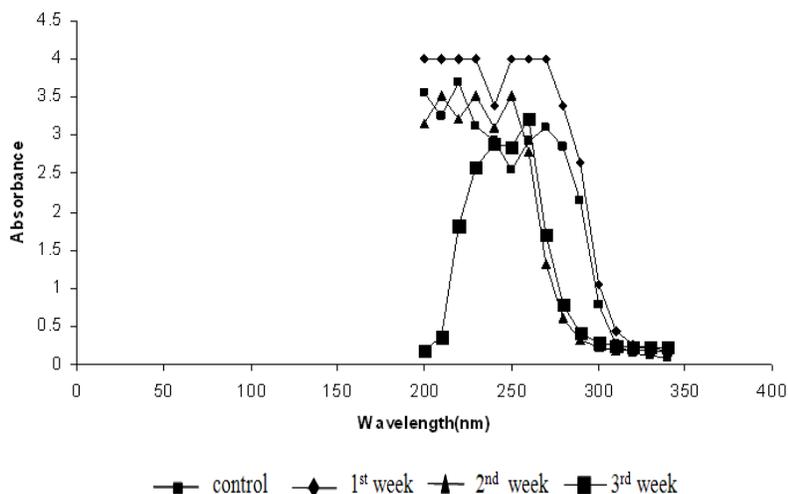


Fig. 2: Decolorization of indigoid Vat Blue 66 by *Pseudomonas desmolyticum* NCIM 2112 grown in minimal liquid base media containing 0.01% glucose Observations were taken place at regular time intervals i.e., after 7 days at 530 nm on UV-vis spectroscope

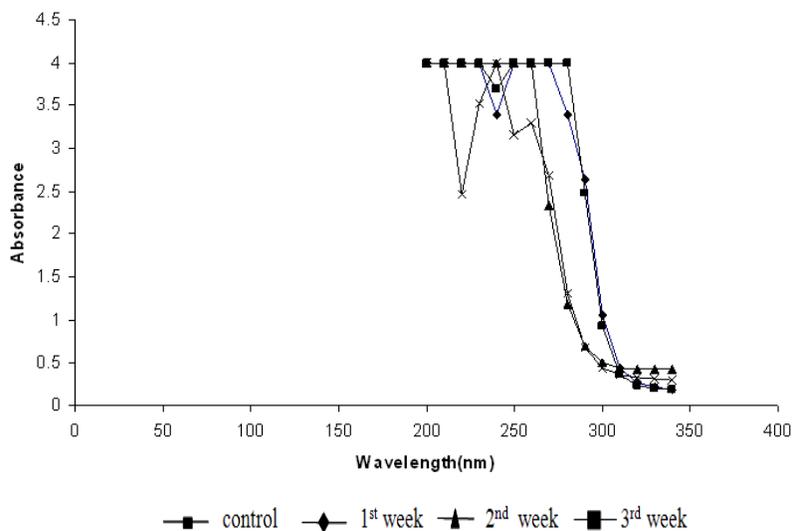


Fig. 3: Decolorization of indigoid Vat Blue 66 by *Bacillus megaterium* NCIM 2087 grown in minimal liquid base media containing 0.01% glucose Observations were taken place at regular time intervals i.e., after 7 days.

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

A - Initial absorbance

B - Observed absorbance

Dyes and chemicals: The commonly used vat dye for cotton dyeing- Vat Blue 66 or Indol Blue CLF 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 ml of microbial suspension containing 120×10^6 cells was inoculated into 100 mL of the above mentioned liquid

medium with glucose and containing Vat Blue 66 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 at 530 nm.

RESULTS AND DISCUSSION

Pseudomonas desmolyticum NCIM 2112 when grown in presence of glucose could decolorize 68% of the dye in 23 days, of which 60% decolorization takes place in 1st week

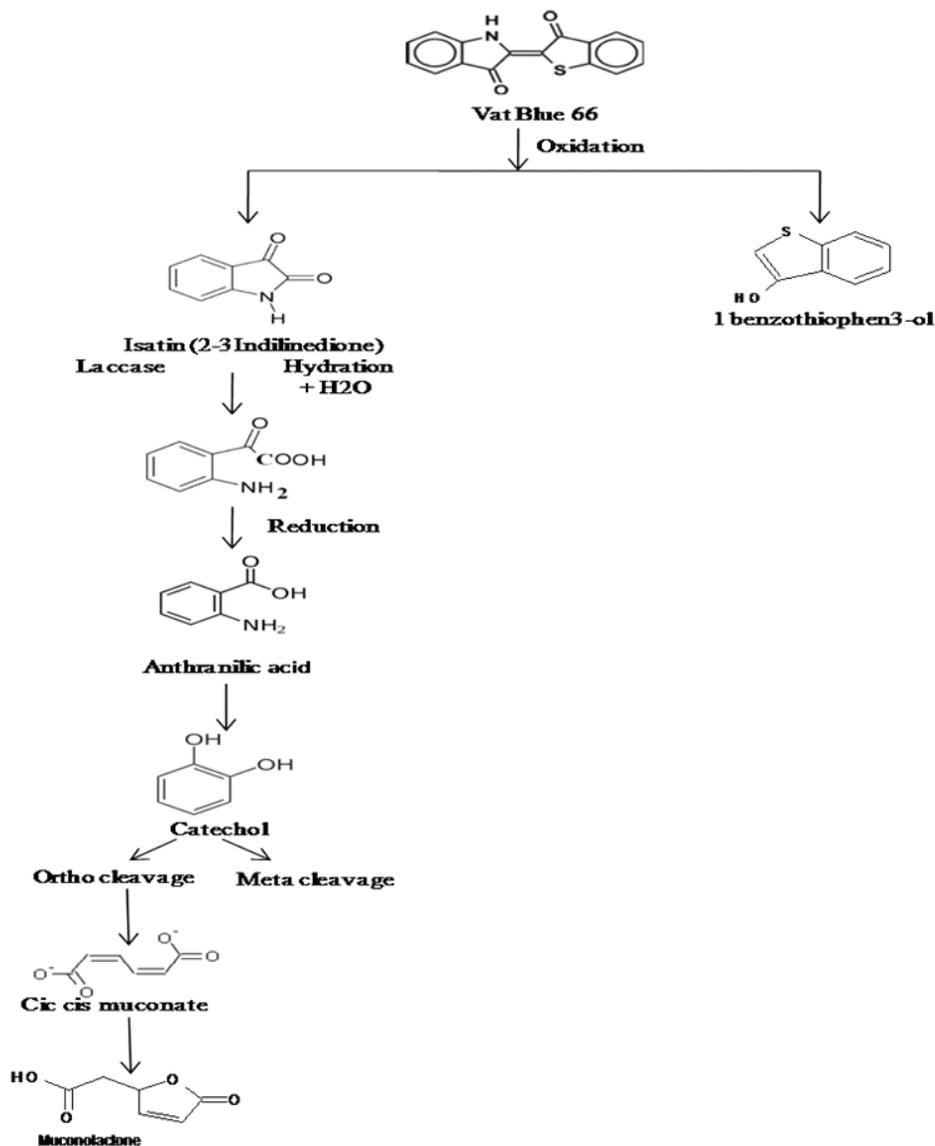


Fig. 4: Proposed pathway for the decolorization of Vat Blue 66 by *Pseudomonas desmolyticum* NCIM 2112

and rest (8%) in remaining two weeks. This is as shown in figure. The compound being colored there are 8 peaks instead of 1 or 2. Similarly, *Pseudomonas desmolyticum* NCIM 2112, in presence of glucose, could bring about 62.5% decolorization in 23 days. The degradation is shown in the (Fig. 2). However, the decolorization is at a much slower rate as compared to that by, *Bacillus megaterium* NCIM 2087. The most probable mechanism of biodegradation is as given in (Fig. 4)

The dye being a colored compound it is showing absorption maxima right from 200 nm to 270 nm. The degradation of the compound by *Pseudomonas desmolyticum* NCIM 2112 (Fig. 2) is more rapid as

compared to that of *Bacillus megaterium* NCIM 2087 (Fig. 3). However, as the compound is being degraded by, in to 2 sets of compounds in the first instance by *Pseudomonas desmolyticum*, as shown in the metabolic routes (Fig. 2) only 4 peaks are maintained at Absorbance 4AU which are at 200, 210, 230 and 250 nm depending upon the degree of unsaturation. The other peaks are at a much lesser absorbance value. At this point the compound becomes colorless. The compounds in the above mentioned figure has been marked as 1 and 2. Then at the end of 14 days of incubation, it can be seen that the absorbance values further decreases of all the peaks indicating that new compounds (like anthranilic acid, and

catechol) are being formed with lesser unsaturation yet retaining the benzene ring nucleus. Finally by 21st day the absorbance reduces further indicating that even these products are getting metabolized as indicated in the above mentioned figure. Now the question arises regarding the intermediate 1-benzothiophene-3-ol which is highly water soluble. This might be remaining in the medium. However, in a natural flowing water body it gets diluted and therefore, has no ecotoxic effect.

In case of *Bacillus megaterium*, the degradation being slow it starts at a much later date during 7 to 14 days. At the end of 21 days 3 out of the 8 peaks are still seen in (Fig. 3) indicating that maybe catechol conversion and degradation is very slow.

Similar results were observed with the degradation of the textile dye indigo with purified laccases from the fungi *Trametes hirsuta* (THL1 and THL2) and *Sclerotium rolfsii* (SRL1) was studied. All laccases were able to oxidize indigo yielding isatin (indole-2, 3-dione), which was further decomposed to anthranilic acid (2-aminobenzoic acid). A possible pathway for the oxidation of indigo to isatin is depicted in Fig. 1. Since the actual catalytic activity of laccases involves the step-wise abstraction (Campos *et al.*, 2001). The end product of the degradation being succinyl CoA. *Aspergillus niger* SA1 could detoxify indole dye-Drimarene Blue by biodegradation (Siddiqui *et al.*, 2010), *Coriolus versicolor* could degrade and detoxify the indole dye Pigment violet 12 (Itoh *et al.*, 1998), *Trametes versicolor* could degrade 2 carpet indole dyes (Ramsay and Goode, 2004). However, there are no reports till date on degradation of dye like Vat Blue 66 which is an indole - oxazole dye and is very toxic in the environment. This being a first report of its kind of microbial detoxification of such a recalcitrant by these 2 organisms belonging to genera *Pseudomonas* and *Bacillus*.

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