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ORIGINAL ARTICLE

BIODECOLOURIZATION OF TEXTILE DYE NEVY BLUE M3 R BY *STREPTOMYCES SPECIES*

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ABSTRACT:

Streptomyces species isolated from the soil of Solapur showed more than 90% decolourization of the textile dye Nevy blue M3R. The decolourization of the dye was due to biodegradation of the dye and it was confirmed by using TLC and HPLC analysis. The effect of different pH and temperature on decolourization of this dye was studied in glycerol aspargine broth. It was observed that the optimum pH for decolurization of dye was pH 7.5 and temperature 45^{o}C. On the basis of morphological, cultural and biochemical characteristic the culture was identified as Streptomyces species.

Key words: *Decolourization,* Nevy blue M3R*, Streptomyces species*

INTRODUCTION

Dye has become an indispensible chemical in many industries like textile, paper printing, colour photographs, pharmaceuticals, food and cosmetics etc. (Mali *et al*., 2000). The major drawback of use of synthetic dyes is it causes water pollution. Effluent from the textile industry is the most polluting amongst all industries, thus seriously requiring suitable treatment technologies (O'Neill *et al.* 1999). It is estimated that 10-15% of these dyes used in textile processing is lost in effluent during the dyeing process (Vaidya and Datye 1982).

If untreated effluent is discharged in the water bodies pose threat to the organisms living in the water body and causes death of the organisms. Some dyes are also carcinogenic and causes cancer.

Treatment of the effluent containing dye is big challenge. Many physico-chemical treatments including aerobic sludge digestion, anaerobic system, membrane filtration, and combination of aerobic and anaerobic system are generally used for the treatment of waste

water. A combined electrochemical and ultrasound technique (sono-electrochemical) was also used for dye decolourization (Rivera, M., *et al.,* 2009).

Treatment of the textile effluent dye with microorganism is cheap and eco-friendly approach. Bacteria, fungi and actinomycetes are having ability to degrade the textile dyes. *Aspergillus niger* and *Phanerochaete chrysosporium* was used for decolourization and degradation of dyes. (Haritha, K. *et al.,* 2009. Actinomycetes are not commonly used in dye decolourization studies. Present study exploits potential of *Streptomyces sp.* for dye decolourization.

MATERIAL AND METHOD:

Collection of soil samples

Soil samples were collected in sterile polythene bags from the textile industries located at Solapur (M.S.). Samples were refrigerated at 4^{o}C till further use.

Source for dye samples

Textile dye Nevy blue M3R was obtained from the Yemule Textile Mills Solapur (M.S.).

Isolation of actinomycetes from soil

Sterile glycerol aspargine agar (GAA) supplemented with antifungal agent Grisofulvin (50 µg/ml) was used for the isolation of the actinomycetes. The soil sample was serially diluted and 0.1 ml of each 10^{-2} and 10^{-3} dilution was aseptically spread over the glycerol aspargine agar media and plates were incubated at room temperature for 6 days. After incubation actinomycetes were selected and preserved on the glycerol aspargine agar slants and maintained at 4^{0}C in refrigerator.

Screening of actinomycetes for dye decolourization

Screening of actinomycetes for dye decolonization was carried out by adding dye Nevy blue M3R in sterile glycerol aspargine agar at concentration 100 mg l^{-1}. Isolated actinomycetes were spot inoculated on sterile plates and incubated at room temperature for six days. After incubation, plates were observed for zone of decolourization surrounding the colonies. The positive actinomycetal isolates were used for further studies.

Identification of the Actinomycetal culture:

Identification of the actinomycetal culture was carried out on the basis of morphology, colony character and microscopic studies.

Determination of absorption maxima (λ max) of dyes

The absorption maxima (λ max) value for the selected textile dye Nevy blue M3R was determined by using U.V.-VIS spectrophotometer (Make- Bioage)

Broth culture studies

Positive culture of actinomycetes (AR28) showing decolourization of the dye was used to study decolorization of dye in broth medium. For this sterile glycerol aspargin broth (GAB) having 100 ppm of dye was used. The culture was inoculated in the flask were kept on the shaker at 120 rpm. After 24 hours sample was taken from the flask aseptically, centrifuged and absorbance of

the supernatant was taken at 622 nm wavelength. Percentage decolourization was determined by the formula

$$\% \text{ decolourization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

Effect of pH on dye decolourization

To check the effect of pH on the dye decolourization, the pH of GAB was adjusted to various pH values viz. 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0. The flasks were inoculated with actinomycetes culture. After incubation percent decolourization at different pH was calculated.

Effect of temperature on dye decolourization

To check the effect of incubation temperature on the dye decolourization, thes flasks were incubated at 25^0C, 30^0C, 35^0C, 40^0C, 45^0C 50^0C 55^0C and at 60^0C separately. The flasks were inoculated with actinomycetes culture. After incubation percent decolourization at different temperatures were calculated.

Thin Layer Chromatography (TLC)

After decolourization the broth was used for the extraction of dye metabolite. The filtered broth was added with equal volumes of ethyl acetate and shaked vigorously. Ethyl acetate layer was separated and evaporated. The crystals obtained after extrations were dissolved in small volume of AR grade methanol and the sample was used for TLC analysis. TLC analysis of pure dye and metabolite was carried out on commercially available TLC silica gel 60 F254 plates (Make- MERCK) using mobile phase solvent system n-propanol, methanol, ethyl acetate, water and glacial acetic acid (3:2:2:1:0.5) and the spots were developed using iodine chamber.

High Performance Liquid Chromatography (HPLC)

The crystals obtained after extraction were dissolved in small volume of HPLC grade methanol. HPLC analysis of pure dye and metabolite was carried out by using Younglin, (Acme 9000) on C18 column (symmetry, 4.6 · 250 mm). The mobile phase was methanol with flow rate of 1.0 ml min^{-1}.

RESULT AND DISCUSSION:

Isolation of actinomycetes from soil

30 actinomycetes were isolated from the soil of Solapur region and labeled as AR01 to AR30 and further used for decolourization studies.

Screening of actinomycetes for dye decolourization

Out of 30 actinomycetes isolated, 09 cultures showed decolourization of the textile dye Nevy blue M3R on Glycerol aspargine agar plate. Maximum decolourization of the dye was carried out by AR28 which was used for the further study. Zhou and Zimmermann (1993)

embarked on larger screening process in which the decolourizing capabilities of 159 actinomycetes were investigated. Positive results were obtained for 83 isolates.

Identification of the Actinomycetal culture:

On the basis of morphology, colony character and microscopic studies, the selected actinomycetes cuture was identified as *Streptomyces sp.*

Determination of absorption maxima (λ max) of dyes

The absorption maxima (λ max) value for the selected textile dye Nevy blue M3R was determined by using U.V.-VIS spectrophotometer (Make- Bioage). The absorption maxima (λ max) value was 622nm.

Effect of pH on decolourization of Nevy blue M3R dye by *Streptomyces sp.*

It was observed that in shaking condition after six days incubation at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0 dye decolourization was 59.1%, 60.02%, 92.95%, 92.99%, 92.97%, 93.9%, 92.65%, 83.9%, 74.0%, 59.7%, 57.98%, 40.9% and 40.08% respectively by *Streptomyces sp.* (Fig. 1)

From these results it was observed that pH ranging from 6.0 to 8.0 showed more than 90% decolourization of textile dye Nevy blue M3R on third day. At pH 7.5 maximum decolourization of dye Nevy blue M3R was observed. At alkaline pH also there was good decolourization. This ability of culture is very useful for application in treatment of effluent with alkaline pH.

Thus it was concluded that pH 7.5 was optimum pH for decolourization of dye Nevy blue M3R by actinomycetal isolate *Streptomyces sp.*

Zang *et al.,* (2003) reported that the low pH favors dye adsorption in case of *Penicillium oxalicum. A. ochraceus* decolorized Reactive blue-25 at different pH ranging from 3–9. (Parshetti *et al.,* 2006).

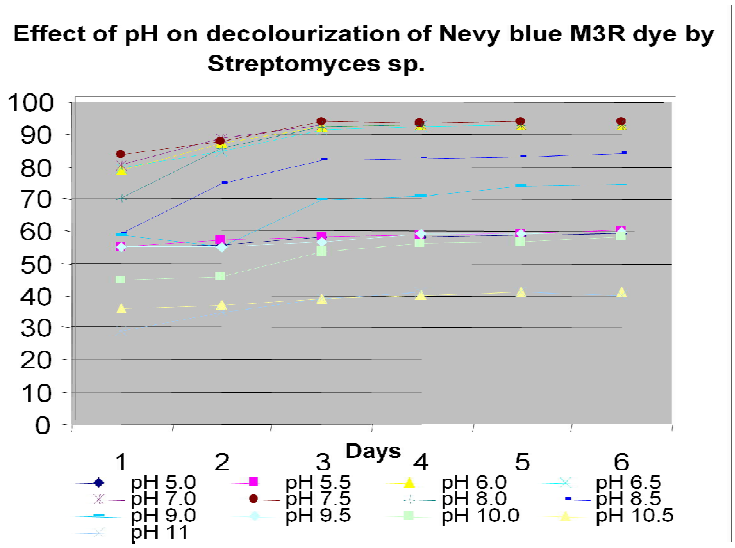


Fig. 1 Effect of pH on decolourization of Nevy blue M3R dye by *Streptomyces sp.*

Effect of temperature on Nevy blue M3 R dye decolourization by *Streptomyces sp.*

It was observed that in shaking condition after six days incubation at incubation temperature 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C decolourization was 50.36%, 61.86% 74.9%, 75.3% 95.7% 78.46 %, 54.87% and 38.6% respectively (Fig.2)

Incubation temperature 45°C showed highest (90%) dye decolourization within three days. From these results it was concluded that the optimum temperature required for the decolourization of Nevy blue M3 R dye by actinomycetal isolate *Streptomyces sp.* is 45°C.

Moosvi S. *et al.,* (2005) studied the effect of temperature on decolourization using RVM 11.1 consortium and it was noticed that with an increase in temperature from 20 to 30°C, the decolourization rate increased and a further increase in temperature to 50°C drastically affected decolourization activity of mixed bacterial culture.

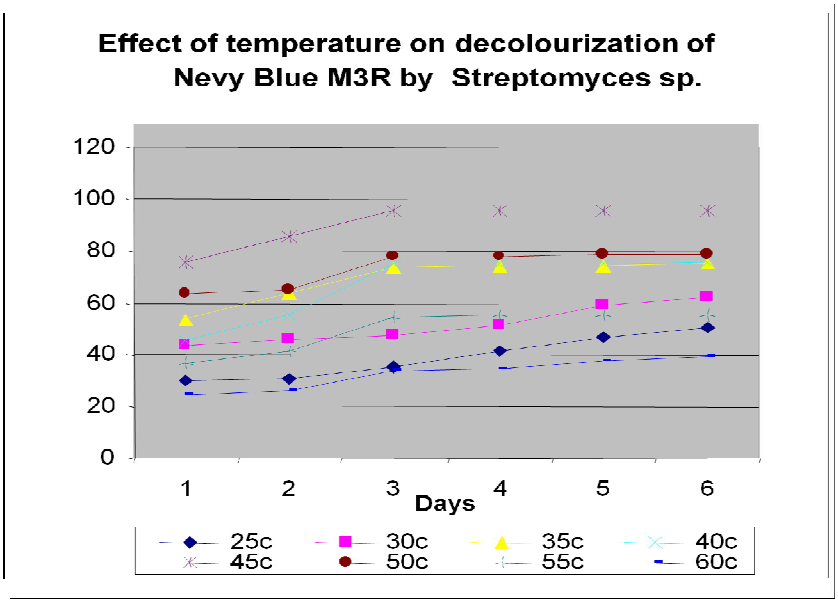
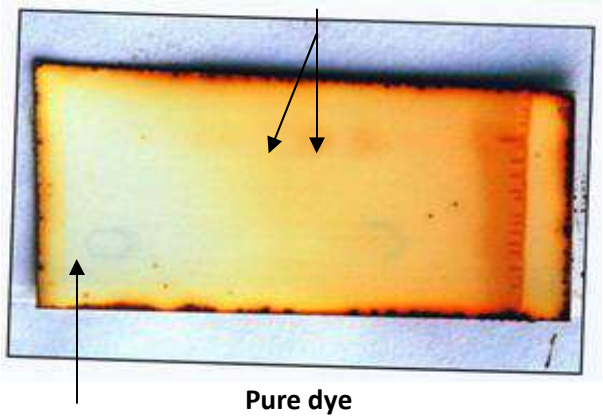


Fig. 2 Effect of temperature on decolourization of Nevy blue M3R dye by *Streptomyces sp*

Thin Layer Chromatography (TLC)

The TLC analysis of the pure dye and the metabolites of Nevy blue M3R dye after degradation showed that the dye Nevy blue M3R was pure and showed only one spot on the TLC plate. The degraded product formed after the action of *Streptomyces sp.* showed two spots on the TLC plate indicating the degradation of the dye**.** (Plate. 1)

Two spots of product.



Pure dye

Plate. 1 Thin layer chromatography of Nevy blue M3 R and its metabolites.

The Rf value of pure dye was 0.77 and of first product was 0.71 and of second product was 0.66. This indicates that there was formation of product by degradation resulted in to decolourization of the dye.

High Performance Liquid Chromatography (HPLC)

HPLC analysis of pure dye Nevy blue M3R revealed that there was presence of only one strong peak (Fig. 3) with retention time1.884 minutes showing area 100%.

From the presence of single peak it was clear that dye used for decolourization studies was pure. (Table1.)

The metabolites formed from pure dye Nevy blue M3R showed three peaks. One peak was showing the same retention time (1.9000) like that of the pure Nevy blue M3R, however the height and area was less indicating decreased concentration of dye. (Fig. 4)

Second peak obtained indicated that it was newly formed metabolite showing retention time 2.6333 minutes with area 47.17 %. The third peak showed retention time 2.8167 with area 35.94% indicating formation of new metabolites after degradation. (Table 2.)

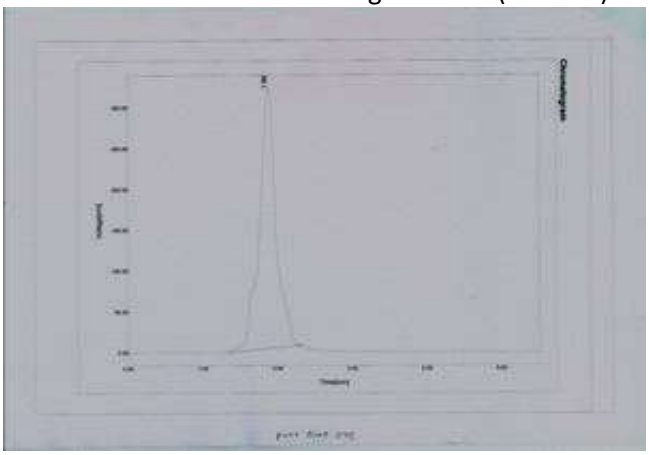


Fig. 3 HPLC analysis of pure Nevy blue M3R dye

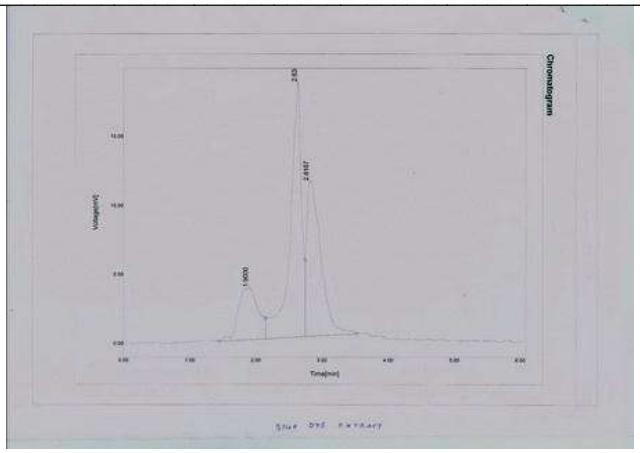


Fig. 4 HPLC analysis of metabolite of Nevy blue M3R dye.

Table. 1. HPLC analysis of pure dye Nevy blue M3R

Sr. No.	Name	Retention Time (RT) Min.	Height (mV)	Area%
1	Pure Nevy blue M3R Dye	1.884	318.5067	100.0

Table 2. HPLC analysis of metabolites of Nevy blue M3R dye.

Sr. No.	Name	Retention Time (RT) Min.	Height (mV)	Area%
1	Nevy blue M3R	1.9000	3.8029	16.89
2	Nevy blue M3R Metabolite 1	2.6333	18.4941	47.17
3	Nevy blue M3R Metabolite 2	2.8167	11.2934	35.94

CONCLUSION-

Streptomyces sp. isolated from the Solapur region was showing great potential in decolourization of textile dye Nevy blue M3 R. It decolourizes more than 90% of dye Nevy blue M3 R Blue 160. The optimum pH for the decolourization of dye was pH 7.5 and temperature was 45^{o}C. The decolourization of the dye was by the degradation of the dye by *Streptomyces sp.* which was confirmed by TLC and HPLC analysis. On the basis of morphological characteristic, microscopic observation and mycelial arrangement, culture was identified to *Streptomyces. sp.* This culture can be used to treat textile effluent containing dye Nevy blue M3 R which is commonly used in textile industry.

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