Screening and Exploration of azo dye decolorizing Actinomycetes from Marine Sediments

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Abstract- Effluent discharge from textile industry into water bodies is currently causing significant health concern to environmental regulatory agencies. Reactive azo dyes have been identified as the most problematic dyes in textile effluents due to their high stability against light, temperature, detergents, chemicals and microbial attacks. The present study was designed for screening and identification of dye decolorizing bacteria from marine Sediments. Marine Actinomycetes were isolated from marine sediments on Maltose Yeast extract agar medium by serial dilution method. The isolated strains were evaluated for their decolurisation ability against reactive Red dye. The optimum pH and temperature on the rate of decolourisation was determined. The effect of carbon and nitrogen sources was determined by supplementing mineral salt medium with different carbon and nitrogen sources.

Index Terms—Marine sediments, Marine actinomycetes, Azo dyes, Decolourisation, Degradation,UV-VIS analysis.*Nocardia sps*.

1 INTRODUCTION

Textile industry is one of the greatest generators of liquid effluent pollutants due to the high quantities of water used in the dyeing process[1]. Dye present in textile waste water cause not only aesthetic problems but also threat to public health. Pollution, due to textile industry effluents, has increased during recent years. Approximately 10,000 different dye and pigments are being used industrially and 0.7 million tons of azodye are being produced annually[2]. Out of all chromogenic textile dyes, the reactive groups of azodyes are predominantly used in the dying process based on the superior fastness for the fabric, high photolytic stability and resistance towards microbial degradation [3][4]. Azodyes have been increasingly used because of their cost effectiveness and wide range of colors when compared with natural dyes [5][6].Most of the liquid & solid effluents from textile industries are treated by physical & chemical methods such as flocculation, adsorption, filtration & oxidation[7].It is very difficult to treat textile industry effluents, because of their high Biological oxidation demand (BOD), Chemical Oxygen Demand (COD), heat, colour pH and the presence of mutations.[8] All the physical and chemical methods used for the remediation of hazardous effluents remains as drawback due to the deposition of sludge[9].

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Bioremediation, an ecofriendly technique may be used as an alternative strategy to lessen the burden of sludge[10]. Thus, the present study was conducted to isolate and screen for azo dye degrading actinomycetes from marine sediment sample.

2 Materials & Methods:

2.1 Materials

The reactive azodyes Congo red was collected from the retail vendors at Madanapalli and Puttur towns of Chittoor district, A.P. India. Nutrient glucose agar (Peptone, Beef extract, Glucose Agar), Maltose (Maltose, Yeast, Agar), starch casein agar (Starch, Casein, Agar, Peptone) were used for the isolation of marine actinomycetes and for the degradation of azo dyes. All other chemicals used were of technical grade.

2.2 Isolation & Characterization of Dye Degrading Marine Actinomycetes:

The marine samples were collected from the sediments of sea shore of Bay of Bengal, at Kotta Kodur, Nellore district, A.P., India. The marine samples were pretreated for the isolation of actinomytes. About 10g of sediment soil sample was added to different media (Nutrient glucose agar, Maltose Yeast agar, Starch casein agar) to screen for marine actinomycetes. The pure actinomycetes strains were inoculated into NGA medium amended with reactive azodyes and the potential degrading isolates were used for further study. The dye degrading strains were identified based on the morphological and biochemical properties

2.3 Decolourization percentage

To etermine the ecolourisation percentage, the Congo red dye

was incubated with A,niger for ifferent time intervals at their respective pH, temperature, 1% of Carbon and nitrogen sources and samples were removed at regular intervals and analyzed for decolourization activity. The percent decolourization of effluent was determined by using the formula.

$$D = \left[\frac{A_0 - A_1}{A_0} \right] X\,100$$

D = Decolourization
A_0 = Initial Absorption
A_1 = Final Absorption

2.4 Effect of physic-chemical factors on dye decolourization:

In order to determine the effect of pH on dye decolourization decolourization efficiency of the isolate, experiments were performed at different pH (5, 7 & 9) of Nutrient Broth medium by keeping other conditions constant (dye concentration 400 ppm). The medium was incubated at different temperatures (15°C, 37°C & 50°C) to check the effect on dye decolourization

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2.5 Effect Carbon & Nitrogen on dye decolourization:

To study the effect of Carbon and Nitrogen on dye decolourization, the Nutrient Broth medium containing 400pm/ml of dye Reactive red was supplemented with 1% Carbon sources (Sucrose & Maltose) and 1% Nitrogen sources (Yeast extract & Peptone) and finally the medium was inoculated with the marine isolate and incubated for 7 days for measuring the extent decolourization and the percentage was calculated as

2.6 Statistical Analysis

All the experiments were conducted in triplicate and the data was analysed for Mean, Standard Deviation and Significance of Variance by using Sigma Stat software.

3.0 Results

In the present study, marine samples were collected and screened for dye degrading actinomycetes based on morphological features 15 different strains were isolated. Among the 15 different isolates, the isolate KN5 showed prominent decolourization efficiency when compared with all other isolates.In order to observe the growth pattern, the isolate KN5 was streaked on to different media. As shown in Fig. 1, the Nutrient glucose agar, Maltose Yeast agar and Starch casein agar proved to be good source for the growth of the isolate KN5.

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2.3 Culture media

Potato Dextrose Agar (PDA) medium and Mineral salt media (MSM) used as selective media for growth of fungal strains for

Fig. 1 Growth pattern off isolate KN5 on Different Media

The KN5 strain was found to be gram+ve and rod shaped. The isolate KN5 was found to be indole –ve, MR +ve, VP +ve, citrate +ve and also +ve towards nitrate reduction (Table 1). The isolate KN5 exhibited Amylase, lipase, Gelatinage and urease activity and melanin production is totally absent (Table 2). Based on the growth pattern on different media, morphological, microscopic, biochemical and enzymatic properties, the isolate was identified as *Nocardia sps* KN5 strain.

Table 1. Biochemical Characteristics of isolate KN5

SL NO	Biochemical test	Result
1	Indole	-ve
2	Methyl Red	+ve
3	Voges-Proskauer	-ve
4	Citrate	+ve
5	Nitrate Reduction	+ve

Table 2. Production of Extra cellular enzymes by KN5.

SL NO	Enzyme Activity	Result
1	Amylase	+ve
2	Lipase	+ve
3	Gelatinase	+ve
4	Cellulase	-ve
5	Melanin	-ve
6	Urease	+ve

3.1 Decolorization potential of Nocardia KN5:

The evaluate the decolourization efficiency of the Nocardia KN5 strain was inoculated in to the nutrient glucose medium amended with Red dye (400ppm) and incubated at different time intervals. The decolourization of the Red dye was increased with increase in the time when compared with control (Fig 2). The results indicated 50%, 70% and 81.09% of decolourization on 3rd, 5th and 7th day of incubation respectively (Fig 3). Relative effectiveness Relative effectiveness of the Nocardia sps (KN5) for the decolourization of Reactive Red clearly indicates that it can be utilized for the removal of Red azo dye from textile effluents.

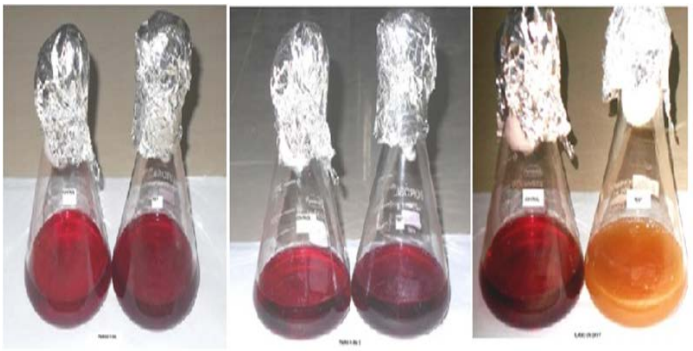

Figure 2: Bioecolourisation of Reactive Red in liquid medium at different intervals by *Nocardia sps* (KN5).

3.2 Effect of Physiochemical Factors on Decolourization

The effect of physicochemical factors on the decolourization efficiency of Nocardia sps (KN5) was studied. The strain was able to decolourize the Red dye at all temperatures ranging from 15°c to 50°C. But, the decolourization efficiency seems to be higher at 37°C and found to be optimum temperature for decolourization of Red dye (Fig 4). The mesophilic temperature is ideal [11][12] since maintaining high temperature is uneconomical for decolourization an degradation at psychrophilic temperature was found to be too low[13][14]. In case of pH as a variable, decolourization was found to be higher at pH 7 whereas decolourization efficiency was decreased both at acidic and alkaline pH at all-time points (Fig 5).From this results it can be concluded that decolourization was optimum at neutral pH and mesophilic temperature [15].

3.3 Types of carbon and Nitrogen sources

The effects of Carbon and Nitrogen sources on decolourization of red colour dye by Nocaria sps (KN5) were evaluated. It was found that maximum decolourisation occurred with1% sucrose (85%) and application of maltose showed no effect on the percentage of decolourization(Fig6).Similarly,the yeast extract induce the percentage of decolourisation (Fig7).

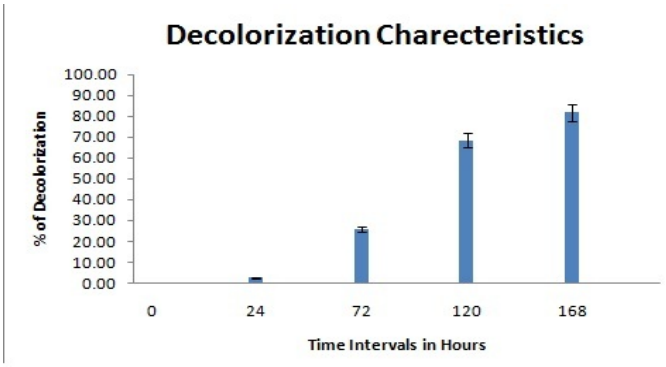

Figure 3: Effect of incubation time on decolourization of Red dye by *Nocardia sps* (KN5).

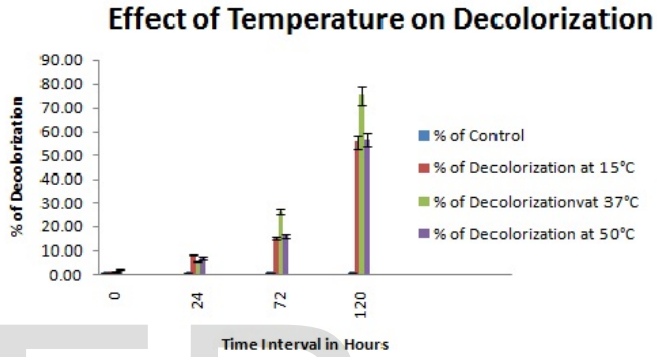

Figure 4: Effect of temperature on decolourization of Red dye by *Nocardia sps* (KN5).

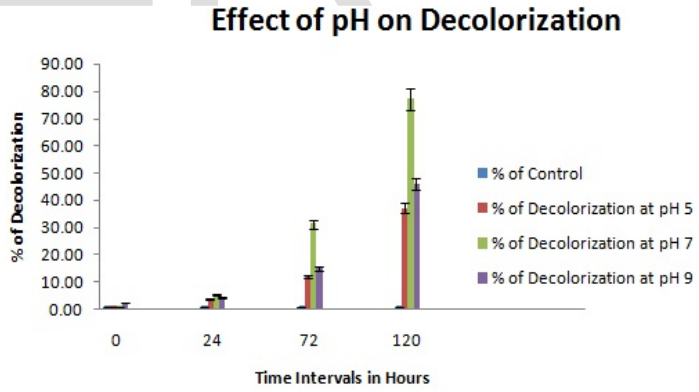

Figure 5: Effect pH on decolourization of Red dye by *Nocardia sps* (KN5).

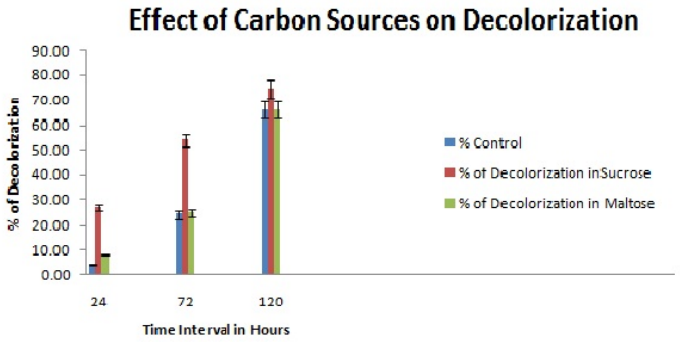

Figure 6: Effect Carbon source on decolourization of Red dye by *Nocardia sps* (KN5).

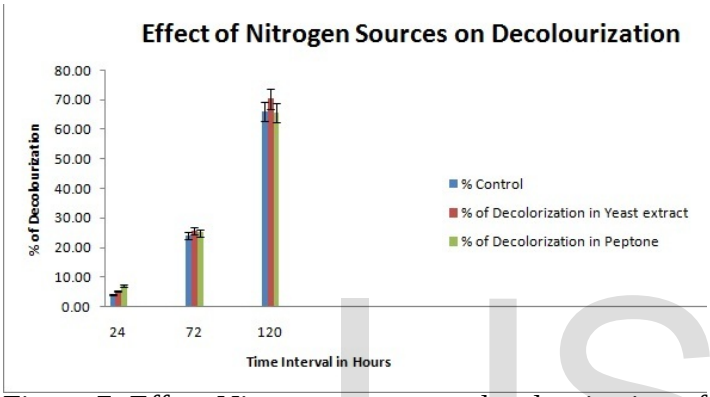

Figure 7: Effect Nitrogen source on decolourization of Red dye by *Nocardia sps* (KN5).

4 Conclusion

The *Nocardia sps (*KN5) was able to completely remove the colour of the azo dye after 7 years of incubation. However, further study is needed to understand the mechanism of bioremediation of Red dye polluted textile waste water.

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