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BACTERIAL DEGRADATION OF CRYSTAL VIOLET DYE

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Abstract- Crystal violet belongs to the family of Azo dyes, which are extensively used in textile industries. Disposal of dyes in the environment causes damage to the ecosystem and biodiversity. Microbial degradation and decolourization of crystal violet is an eco-friendly and sustainability enhancing process. In the present study, crystal violet degrading bacteria were isolated from textile effluent on Nutrient agar amended with the dye. 11 potent bacterial strains were selected, which exhibited the maximum decolourization in 48 hours. Kinetic studies were performed to analyse the influence of different dye concentrations, pH and temperature on degradation rate, under static conditions. All the 11 bacterial strains could decolourize 500 µg of crystal violet within 24 hours and could grow in media containing 1000 µg of crystal violet. Maximum degradation was observed in the pH range of 6-9, with optimum temperature as 37°C. The organisms were identified using vitek system and were found to be belonging to *Stenotrophomonas maltophilia* and *Enterobacter cloacae complex*. Thus, the study emphasizes the potential role of microbes for rapid and efficient degradation of azo dye, thus also aiding safe discharge of textile effluent.

Key words - Crystal violet, Azo dye, dye degrading bacteria, textile effluent, *Stenotrophomonas maltophilia*, *Enterobacter cloacae*.

I. INTRODUCTION

Over the last few decades, globalization and industrialization have drastically increased, which has led to an increase in environmental pollution. Among various industries, the textile dying industries discharge a large volume of waste water after the dyeing process [M. Sudha et al, 2014]. It is estimated that around 10 -15% of the dyes are lost in the effluent during the dyeing processes [M. Sudha et al, 2014]. This wastewater contains different types of dyes, such as azo, diazo, acidic, reactive, basic, disperse, anthraquinone dyes.

Crystal violet is a type of azo dye that is extensively used in the textile industry. It is a cationic dye, also known as hexamethyl pararosaniline chloride, with the molecular formula $C_{25}H_{30}N_3Cl$ [Chung et al. 1983]. It also has extensive applications in human and veterinary medicine as a biological stain [S. Adeeb Mujtaba Ali et al, 2014]. Azo dyes have a synthetic origin and a complex aromatic structure (N=N group). Depending upon the number of azo-groups, azo dyes are divided into monazo, disazo, trisazo, tetrakisazo and polyazo. These dyes account for around 60-70% of all dyes used in food and textile manufacture [Saratale et al., (2013), S. Kannan et al, 2013].

Discharge of crystal violet and other azo dyes in the environment causes serious health and environmental problems. It may affect photosynthetic activity in aquatic life by reducing light penetration. This also alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations [S. Kannan et al, 2013]. It greatly affects the water quality. The high concentration of dyes

is known to cause ulceration of the skin, and mucous membrane, dermatitis, perforation of the nasal septum, severe irritation of the respiratory tract and on ingestion may cause vomiting, pain, haemorrhage, and sharp diarrhoea [C. Lavanya et al., 2014].

Various physico-chemical methods are employed for the decolourization of azo dyes. Although they can remove dyes partially, various limitations prevent them to be economical and thus cannot be used widely and economically [S. Kannan et al, 2013]. Biological methods have gained more attention recently because they are eco-friendly and economically feasible. A number of microorganisms have been found to be able to decolourize textile dyes, including bacteria, fungi, and yeasts [S. Kannan et al, 2013]. Bacteria capable of dye decolorization, either in pure cultures or in consortia, have been reported [Telke et al. 2008; Kalyani et al. 2009]. Bacterial azo dye biodegradation proceeds in two stages. The first stage involves reductive cleavage of the dyes, azo linkages, resulting in the formation of generally colourless but potentially hazardous aromatic amines. The second stage involves degradation of the aromatic amines. Azo dye reduction usually requires anaerobic conditions, whereas bacterial biodegradation of aromatic amines is an almost exclusively aerobic process [Alabdraba et al.,2014]. Enzymes catalyzing azo dye reduction may either be specialized enzymes (catalysing only the reduction of azo dyes) or non-specialized enzymes (non-specific enzymes that catalyze the reduction of a wide range of compounds). The predominant enzymes are azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases.

The present study focused on isolation and screening of degraders from dye textile effluent for decolourizing crystal violet (Azo dye) and optimize various parameters to maximize the decolourization.

II. MATERIALS AND METHODS

Sample collection and screening for dye degraders: A textile dye effluent sample was collected in a clean container from Genesis Industry, Tarapur, Maharashtra. The effluent was used to carry out enrichment in the nutrient broth method containing 10 ppm of crystal violet. After enrichment of 15 days the broth was cultivated on the nutrient agar medium amended with 30 ppm crystal violet dye and incubated at 37°C for 24 hours. The bacterial colonies which exhibited decolourizing ability were chosen for further studies. More such studies were carried out with 100 ppm and 500 ppm of crystal violet. Thus, 11 isolates that gave the best degradation at 500 ppm concentration of crystal violet after 48 hours were screened and selected for kinetics study.

Kinetic Studies

Effect of dye concentration: To determine the effect of different concentrations of crystal violet dye decolourization, nutrient broth media was amended with different concentrations of crystal violet to get a final concentration of 500, 750 and 1000 ppm. The inoculated media in the flasks were incubated for 48hrs at 37°C and degradation activity was analysed [S. Kannan et. al., 2013].

Effect of pH: The pH of nutrient broth media was adjusted to 4, 6, 7.4 and 9 and then further inoculated with test strains to study its effect on decolourization of crystal violet (500 ppm concentration) at 37°C for 48hrs [S. Kannan et. al., 2013].

Effect of temperature: To study the effect of temperature on decolourization of crystal violet dye, the nutrient broth media containing 500 ppm concentration of crystal violet was inoculated with test strains at room temperature and 37°C for 48 hrs [S. Kannan et. al., 2013].

Decolourization assay: The analysis of the crystal violet dye degradation by test strains was carried out by absorption studies, which indicated the decolourization of the dye in the broth. To quantify the decolorization rate under different environmental conditions, the samples after 24 hours and 48 hours of incubation were withdrawn and centrifuged at 10,000 rpm for 5 min. The supernatant was collected (diluted if required) and was read at 540 nm (maximum wavelength) by colorimeter [S. Kannan et. al., 2013]. The percentage of decolorization was calculated by the formula-

Percent Decolourization = (Initial absorbance value–Final absorbance value / Initial absorbance value) × 100.

Identification of the dye degraders: Identification of the dye degraders was done by VITEK METHOD and by studying the morphological characteristics.

III. RESULTS AND DISCUSSION

Screening for potent strains will be beneficial to treat waste water from textile industries. In the current study, the agar plate assay was carried out to detect the decolorization activity of the enriched strains obtained from textile waste water samples. The pure cultures of the 11 isolates were selected as test strains on the basis of their dye degradation ability on nutrient agar plates amended with 500 ppm of crystal violet (fig. 1).

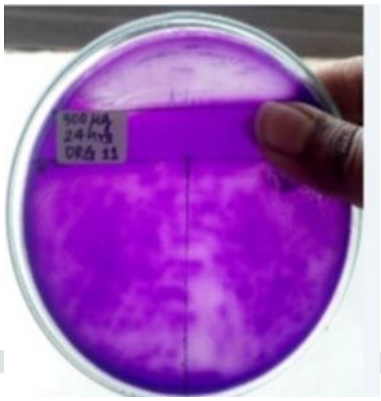

Fig 1. Isolate no. 11 exhibiting crystal violet dye degradation

The broth assay was carried out to detect the optimum conditions for decolorization by the test strains. The culture suspensions of the selected 11 isolates (O.D. 0.1 at 540nm) were inoculated into the nutrient broth containing varying concentrations of crystal violet (500, 700 and 1000 ppm) and incubated appropriately for degradation assay studies. After 48-hour incubation, the media samples were collected, centrifuged, and their optical density was measured at 540 nm. The decolorization percentage was calculated and the graphs were plotted.

All the 11 isolates could decolourize crystal violet in a concentration range of 500 ppm to 1000 ppm. Maximum decolorization was observed at 500 ppm of crystal violet by all isolates. A maximum decolorization percentage of 99.24% was observed within 48 hours by isolate 5, followed by 97.72 by isolate 2. All the 11 isolates could decolourize dye concentrations of 1000 ppm in 48 hours. The maximum decolorization of the crystal violet dye was exhibited by isolate 11 (51.40% degradation), within 48 hours. The decolorization abilities of microorganisms can be enhanced by gradually exposing them to higher concentrations of synthetic organic chemicals. The adaptation of microorganisms to toxic and high concentration compounds was found to be improving the rate of decolorization. From a previous study, it was found that the bacterial dye degraders could decolourize 97.12% dye of 300 ppm concentration in 24 hours [S. Kannan et. al., 2013]. Comparatively, from the present study, a degradation of 99.24% was observed for 500 ppm concentration in 48 hours.

Fig 2. Isolate no. 02 exhibiting crystal violet dye degradation by tube method

A pH range of 4 to 9 was used for the kinetic study. Optimum pH for all isolates was found to be in the range of pH 6-7.4. The decolorization activity showed a decrease at pH below 6 or above 8. However, the degradation activity being functional at both acidic and alkaline pH indicated the capability of the organisms to carry out degradation in diverse conditions. Similar results were observed in previous studies reported. The optimal pH

for dye degradation was found to be between 6.0 and 10.0 [Chen, et al., 2007]. In another study, the maximum decolorization of azo dye was achieved at pH 7.0 with 93.23% degradation in 48 hours [S. Kannan, et al., 2013].

Table 1. Effect of pH on crystal violet decolourization

ORGANISM	% DECOLORIZATION (48 HOURS)			
	pH 4	pH 6	pH 7.4	pH 9
1	40.90	70.40	50.75	43.10
2	43.40	74.20	97.72	67.40
3	40.10	84.80	96.96	75.00
4	43.90	71.90	96.21	61.30
5	40.10	38.60	99.24	35.60
6	43.10	67.40	73.40	63.63
7	42.40	79.50	78.70	65.90
8	37.10	80.30	83.30	62.87
9	40.15	78.70	67.40	60.60
10	52.20	79.50	78.03	65.90
11	49.20	80.30	79.50	73.48

The degradation of crystal violet was more efficient at 37℃ in comparison to room temperature. However, the difference was not statistically significant (p-value>0.05). The organisms were identified using the vitek system and were found to be belonging to *Stenotrophomonas maltophilia* and *Enterobacter cloacae complex*.

IV. CONCLUSION

The isolation of efficient dye decolourizing bacteria from the textile effluent water directs the natural adaptation of the microorganisms to survive in the presence of toxic dyes. Since these microorganisms do not need any additional requirements for adaptation in the presence of toxic dyes, they are beneficial in the treatment of textile effluent. All the 11 isolates could decolourize the dye efficiently up to 1000 PPM concentration in 48 hours under optimum static aerobic conditions, indicating that further studies can be carried out to build a robust consortia of dye degraders. Thus, it can be concluded that these isolates can be used for effective biological treatment of textile effluent.

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VI. CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests with whomsoever.

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