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Biodegradation of synthetic dyes by *Bacillus subtilis* under static condition

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Due to industrial development, the amount and variety of hazardous substances added to the environment has increasing drastically. A significant number of synthetic compounds which are not related to natural ones persist in the environment and create health hazardous for human beings. *Bacillus subtilis* was isolated from Match box industrial effluent disposal site. It was characterized and exploited for its dye degradation potential on synthetic dyes (Acid scarlet 3R Conc., Brown Ex – 399, Crystal violet, Green G Conc., Swiss pink) under static condition dose.

Key words : Synthetic dyes, *Bacillus subtilis*, Bioremediation,Decolourisation

INTRODUCTION

Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Among the biological techniques, it has evolved as the most promising one because of its economical, safety and environmental features, since organic contaminants become actually transformed and some of them are fully mineralized by this technique (Vaidya and Datye, 1982). The bioremediation process involving the use of microbes to detoxify and degrade environmental contaminants has received increasing attention as an effective biotechnological approach to cleanup a polluted environment (Khan and Anjaneyulu, 2005).

Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetics and other industries. Approximately 10,000 dyes and pigments are industrially used and over 0.7 million tons of synthetic dyes are produced annually worldwide (Mc Mullan *et al*., 2001). During processing up to 15% of used dye stuffs are lost in industrial effluents (Vaidya and Datye, 1982; Wong and Yu, 1999). Major classes of synthetic dyes used are azo, anthraquinone and triphenyl methane. In addition to their visual effect and adverse impact in terms of chemical oxygen demand (COD), many synthetic dyes show their toxic, carcinogenic and genotoxic effects (Michaels and Lewis, 1985; Chung *et al*., 1992).

Conventional waste water treatment plants are unable to perform a complete dye removal, 90% of reactive textile dyes persist after activated sludge treatment (Pierce, 1994). Other physico-chemical methods of waste water decolourisation have short comings due to high costs and operational problems with less efficiency. Now-a-days, effective biological process would be of great value, due to their inexpensive, eco friendly nature and lesser sludge producing properties.

A number of biotechnological approaches have been suggested by recent research as of potential interest towards combating pollution by dyes in an eco friendly manner. They include the use of bacteria and fungi often in combination with physico -chemical processes.

Dyeing factory effluent that alters the color and quality of water bodies has been proved to be hazardous to aquatic organisms (Khan and Jain, 1995). Toxic compounds from the dye effluent get into aquatic organisms, pass through food chain and ultimately reach man and cause various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc.

Biological treatment of textile effluents may be either aerobic, anaerobic or a combination of both, depending on the type of microbe being employed (Keharia and Madamwar, 2004). The ability of microorganisms to decolorize and metabolize dyes has long been known and the use of bioremediation based technologies for treating textile waste water has attracted interest (Mc Mullan *et al*., 2001). Several microorganisms have been found to decolorize and mineralize textile dyes. The present study was undertaken to evaluate the effectiveness of *Bacillus subtilis* for the degradation of synthetic dyes.

MATERIALS AND METHODS

Textile dyes :

The commercially available textile dyes such as Acid

scarlet 3R Conc., Brown Ex 399, Crystal violet, Green G Conc. and Swiss pink were purchased from the local market and used for this study without any further purification.

Isolation and screening of microorganisms :

Bacillus subtilis was isolated from the soil of the effluent disposal site of match box industry located in Kovilpatti, Tamil Nadu. Serial dilution method was performed for isolation of the decolourizing bacteria. The morphologically distinct and effective decolourizing *Bacillus subtilis* was screened by inoculating microorganisms into Muller Hinton broth and subsequently subcultured by streak plate technique. The pure culture of the bacterial isolate was maintained in Muller Hinton Agar medium for further analysis.

Biodegradation analysis :

Dye disappearance was determined spectrophotometrically by monitoring the absorbance at maximum wavelength. Absorbance of the supernatant withdraw at different time intervals were measured at the maximum absorption wavelength (λ max) at 509 nm for Acid scarlet, 450 nm for Brown, 592 nm for crystal violet, 618 nm for Green and 531 nm for Swiss pink in the visible region on spectrophotometer (UV). The percentage of decolourisation (Saval, 2003) was calculated as follows :

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance - observed absorption}}{\text{Initial absorbance x 100}}$$

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Dyw decolourisation study :

Soil samples were collected from a polluted site from a match box industry and screened for dye decolourisation organisms. The organism used for decolourisation studies was identified as *Bacillus subtilis*. Decolourisation activity was determined by monitoring the decrease in absorbance. Decolourisation of various synthetic dyes by the growing cells of *Bacillus subtilis* was shown in Table 1. 100% decolourisation took place in 48 hrs after incubation with Swiss pink dye (Fig. 1 C). In Crystal violet (Fig. 1 B) maximum decolourisation was 67.83% after 6 days incubation. It may be due to the difference in chemical structure of both the dyes. Swiss pink has two dimethyl groups in two side chains, whereas Crystal violet has three dimethyl groups in the side chains. It may be the reason that the decolourisation of Crystal violet dyes took longer time. Acid scarlet (81.14%), Brown Ex – 399 (74.25%) and Green (98.08%) were decolorized by the cells of *Bacillus subtilis* after 6 days (Fig. 1 C, D and A).

Table 1 : Per cent decolourisation of various dyes by *Bacillus subtilis* under static condition at room temperature

Days	Acid scarlet	Crystal violet	Brown	Green	Swiss pink
1.	32.00	01.89	40.59	7.20	15.08
2.	43.83	11.70	51.19	27.60	100.00
3.	57.34	51.89	62.62	32.04	-
4.	63.89	56.29	65.84	41.16	-
5.	72.71	67.37	66.88	60.52	-
6.	81.14	67.83	74.25	98.08	-

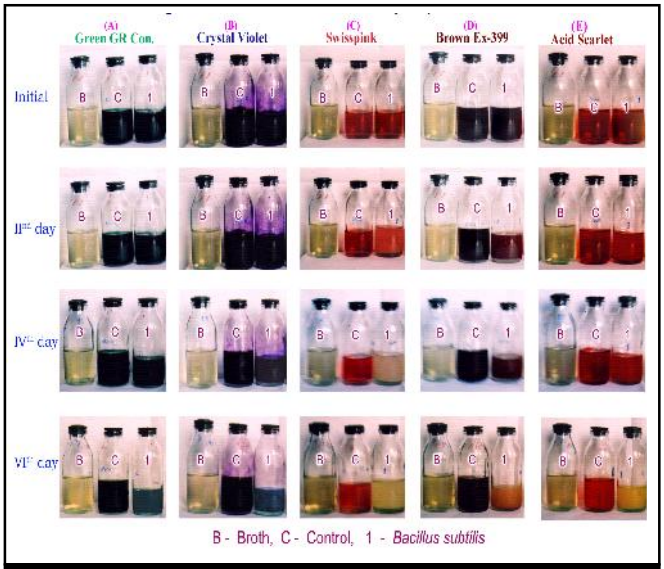

Fig. 1 : Decolirisation of various dyes by *Bacillus subtilis*

Dye decolourisation may take place into two ways either by adsorption or degradation of dyes by the cells. Dye adsorption may be evident from the inspection of the bacterial growth, these adsorbing dyes were deeply colored where as those carrying and degradation will remain colorless. With the isolated bacterial species no cells were towards to be colored with any one of the dyes after transformation. Hence, it was thought to be degradation. Similar to this observation Kwasniewska (1985) demonstrated the oxidative red yeasts *Rhodotorulae* sp., and *Rhodotorulae rubra* had a high

biodegradation against the Crystal violet, and Victoria blue was slowly to degrade by the growing cells of *Bacillus subtilis*. Crystal violet was also decolourised by C*oriolus versicolor, Junalia forgii, Lactiporus sulphureus* and *P. chrysosporium* (Yesilada *et al.*, 2003). The next step should be the design and scaling up of efficient Tailor made biotechnological treatments for industries effluent containing synthetic dyes.

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