

## A NOVEL TEXTILE DYE DECOLOURISING BACTERIA- A REPORT

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**Abstract:** A Novel bacterial sp namely *Kurthia* were isolated from textile dye effluent. The ability of isolated *Kurthia* sp to decolorize the three triphenyl methane dyes namely Crystal violet, Malachite green, Brilliant green, Triarylmethane dyes, Bromothymol blue, Basic fuchsin, phenol red were observed with lower and higher concentration of dyes. The decolourization studies were performed on hourly basis and daily basis and Dissolved Oxygen values were estimated for *Kurthi* sp and they are treated and untreated synthetic dye solutions. It was observed that complete decolourization occurred within 48 hours with 10ppm and 20ppm of the three dyes. 0ppm and 40 ppm of Crystal violet was degraded upto 98% and 94%, respectively in 72 hours. 30ppm of Malachite green and Brilliant green were degraded 100% within 48 hrs. 40ppm of Malachite green and Brilliant green were decolorized by 98% and 96% respectively in 48 hrs. It was observed that 100% decolourization of Crystal violet of 1ppm, 2ppm, 3ppm and 4 ppm concentration occurred with 45, 90, 135, and 180 minutes, respectively. Complete decolourization was noted in Malachite green and Brilliant green for concentration within time interval of 45 to 225 min. 6ppm of both Malachite green and Brilliant green was decolorized by 98% within 270 minutes. The Dissolved Oxygen values of Malachite green and Crystal violet controls were 5.6ppm, 5.4ppm, 5.5ppm respectively. The Dissolved Oxygen levels of corresponding *Kurthia* treated cell free filtrate were 7.3ppm, 7.4ppm and 7.3ppm.

**Key Words:** Textile dye, *Kurthi* sp, triphenyl methane dyes, decolourization, Dissolved Oxygen

### Introduction

Water pollution control is currently one of the major areas of scientific activity. Environmental regulation in many countries have made it mandatory to decolorize dye waste water prior to discharge into neighboring water bodies and waste treatment systems. Dyes such as triphenylmethane dyes, triarylmethane dyes, azo dyes, anthroquinone dyes and reactive dyes are released into the environmental in industrial effluents from tow major sources, the textile and the dye – stuff industries. It is reported that 10 – 20 % of dyes are lost to waste water as a result of inefficient dyeing process.

Triphenylmethane dyes, Triarylmethane dyes are used extensively in textile industries for dyeing nylon, polyacrylonitrile modified nylon, wool, wool, silk and cotton. Paper and leather

industries are also major consumers of triphenylmethane dyes. Food and cosmetic industries also use different types of triphenylmethane dyes and triarylmethane dyes.

Presently most of the processes used for the treatment of dye waste are physiochemical processes such as adsorption, chemical precipitation and flocculation, oxidation by chlorine, hydrogen peroxide and ozone, electrolysis, reduction, electrochemical treatment and ion-pair extraction. These methods are costly, less efficient and produce large amount of sludge. Therefore a currently biological process has achieved greater attention as they are cost-effective, environmentally friendly and does not produce large quantity of sludge.

Biological decolorization of triphenylmethane dyes are widely reported in literature using bacteria, fungi, yeasts and actinomycetes. Most of the studies were carried out on crystal violet.

*Pseudomonas*, *Pseudomallei* BNA, *Mycobacterium* sp., *Corynebacterium* sp., and *Bacillus subtilis* are major bacterial species reported to degrade triphenylmethane dyes. Decolorization of these dyes is exhibited by several fungi such as *Phanerochaete chrysosporium*, Capalash, N., Sharma, P. (1992) *Coriolus versicolor*, *Cyathus bulleri*, *Cyathus striatus* and *Cyathus stercoreus*. Actinomycetes like *Nocardia globerula* and *Nocardia corallina* and yeasts such as *Rhodotorula rubra* were also capable of degrading triphenylmethane dyes.

The biological decolorization of textile dye waste water was reported using aerobic-anaerobic sequential batch or continuous flow reactors (Ganesh et al., 1994). The decolorization of three triphenylmethane dyes namely Crystal violet, Bromophenol blue and Malachite green. *C. bulleri* was found to be most efficient in decolorization. *C. bulleri* has been found to be capable of decolorizing the crystal violet (Vasdev et al., 1995).

The decolorization of crystal violet was reported using three white rot fungi such as *Coriolus versicolor*, *Fumaria troglodytes* and *Phanerochaete chrysosporium* and one brown rot fungus *Laetiporus sulphureus*. It was also reported that crystal violet was oxidized by commercial horseradish peroxidase. A significant rate of oxidation was observed only when hydrogen peroxide was present. Without hydrogen peroxide the enzyme had no effect on the decolorization of dye, thus suggesting that a peroxidase enzyme is probably involved in the oxidation of the dye (Yatoma et al., 1995).

It was observed that different types of microorganisms such as bacteria, actinomycetes, yeasts and fungi can decolorize and degrade different types of triphenylmethane dyes. However, reports on complete mineralization is very much scanty. There is no report on reduction of biochemical and chemical oxygen demand after decolorization. (Azmi et al., 1998)

*Kurthia* spp was selected on the basis of rapid decolorizing activity. Under aerobic conditions, 98% color was removed intracellularly by this strain. A number of triphenylmethane dyes such as Magenta, Crystal violet, Pararosaniline, Brilliant green, Malachite green, Ethyl violet and textile and dyestuff effluent were used in this study. The rates of decolorization of

Magenta(92%), Crystal violet (96%), Pararosaniline (96%), Brilliant green (100%) and Malachite green (96%) were found to be more than that of Ethyl violet (8%) (Sani et al., 1998). The *Caulobacter vibrioides* was discovered to degrade azo dyes (Mazumder 1999).

It was found that *Kurthia spp* had a great potential to transform different triphenylmethane dyes including synthetic and textile dyestuff effluent to colorless compound. In the case of crystal violet, the COD of effluent was reduced by 94.7% with Malachite green the COD of effluent was reduced by 93.2% and with Brilliant green the COD was reduced by 88.2% (Azmi et al., 1999).

*Kurthia spp* can be used as a decolorizing agent and for the removal of toxicity from effluent containing triphenylmethane dyes (Sani et al., 1999). It does not exhibit extracellular decolorizing activity. Cells are responsible for the biotransformation of Gentian violet or Leucogentian violet (Sani et al., 1999). The rate of decolorizing of triphenylmethane dyes by *Kurthia spp* was higher in comparison to other organisms. Moreover the biotransformation products of Crystal violet by all the strains were the same (Sani et al., 1999).

*Kurthia spp* may be promising bacteria to depollute the effluent containing triphenylmethane dyes. This strain can also decolorize synthetic effluent containing a mixture of different triphenylmethane dyes and textiles and dyestuff effluent (Sani et al., 1999).

## **Material and methods**

### **Isolation of *Kurthia spp***

#### **Collection of Sample**

Various effluent samples were collected from a textile dyeing industry, near cellular at Madurai city.

#### **Serial dilution**

The effluent samples were serially diluted making 10 fold dilutions, 1ml of effluent sample was mixed with 99ml of sterile distilled water, in the conical flask, which gives dilution of 1/100. The dilutions procedure was performed for up to dilution of 1/1000000. The dilution samples were used for the isolation of *kurthia spp*.

#### **Primary culture technique**

Banerjee medium was prepared with crystal violet at 2.5µg/ml concentration and poured in the petriplate at aseptic conditions. 0.1ml of each diluted sample were spread over the surface of the plate using a sterile glass rod and incubated at 30<sup>0</sup> c for 24 hrs. After incubation, the results were observed. Organisms were selected on the basis of clear zone on agar plate.

The isolated colonies were streaked on yeast nutrient agar plates and incubated at 30° c for 24 hrs. Banerjee broth 50ml was prepared aseptically and a single well isolated colony was inoculated into the conical flask.

### **Characterization of samples**

The samples were collected and processed according to the standard microbiological methods under complete aseptic conditions. The samples were inoculated on the appropriate media and incubated at 37° c under aerobic conditions for 24-48 hrs. The isolates were examined for colony morphology, pigmentation, cell shape and gram reaction as per the standard procedure.

### **Analysis of Degradation by *Kurthia spp***

#### **Triphenylmethane dyes**

##### **1. Analysis of degradation on day-basis:**

Cells of *Kurthia spp* was harvested from 24 – 48 hrs culture, were washed thoroughly with 0.1M phosphate buffer with pH 7. 1g/100ml of washed cells were used for decolourization of crystal violet of varying concentration (10ppm, 20ppm, 30ppm, 40ppm) and incubated at 30c. Results were observed for decolourization on a daily basis. Every 25hrs 1ml sample was taken out aseptically and the residual dye in the mixture was extracted with water saturated 1-butanol (2ml). The absorbance of the residual dye extract was measured at the  $\lambda$  max of the dye against 1-butanol. For control only broth (1ml) with dye concentration (10ppm, 20ppm, 30ppm and 40ppm) was extracted with 1-butanol (2ml). The same procedure was repeated for Malachite green and Brilliant green.

##### **II Analysis of degradation on hour- basis**

1/100ml of washed cells were used for decolourization of crystal violet of varying concentration (1ppm, 2ppm, 3ppm, 4ppm) and incubated at 30° c.

Results were observed for decolourization within a day. Every 45hrs 1ml sample was taken out aseptically and the residual dye in the mixture was extracted with water saturated 1-butanol (2ml). The absorbance of the residual dye extract was measured at the  $\lambda$  max of the dye against 1-butanol. For control only broth (1ml) with dye concentration (1ppm, 2ppm, 3ppm and 4ppm) was extracted with 1-butanol (2ml). The same procedure was repeated for Malachite green and Brilliant green. The same procedure was repeated for Malachite green and Brilliant green.

##### **III Estimation of dissolved oxygen using electronic water analyzer:**

For each triphenylmethane dyes, experiments were carried out in 100ml flask having 0.5g of cell mass of *Kurthia spp*. In a total volume of 50ml. Dyes were added with constant stirring at

30°C and incubated until completed decolourization occurred. After this, the cells were separated and Dissolved oxygen values of due controls were also estimated and the reading were tabulated.

### Analysis of degradation by *Kurthia sp*

#### Triarylmethane dyes:

#### I Analysis of degradation on Day-basis:

Cells of *Kurthia spp* was harvested from 24 – 48 hrs culture, were washed thoroughly with 0.1M phosphate buffer with pH 7. 1g/100ml of washed cells were used for decolourization of crystal violet of varying concentration (10ppm,20ppm,30ppm,40ppm) and incubated at 30°C. Results were observed for decolourization on a daily basis. Every 25hrs 1ml sample was taken out aseptically and the residual dye in the mixture was extracted with water saturated 1-butanol (2ml). The absorbance of the residual dye extract was measured at the  $\lambda$  max of the dye against 1-butanol. For control only broth (1ml) with dye concentration (10ppm,20ppm,30ppm and 40ppm) was extracted with 1-butanol (2ml). This procedure was repeated for Basic Fuchsin and Phenol red.

#### Decolorization can be calculated as follows:

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

#### Result

The organism isolated from textile dye effluent may be *Kurthia spp*. Surface colonies on yeast nutrient agar was usually rhizoid and had “medusa-head” appearance under low magnification. The isolated *Kurthia* was completely investigated by microscopic and biochemical examination. Dye degradation activity was measured by using various concentrations of synthetic dye solution prepared in our laboratory. The dye used were Crystal violet, Malachite green, Brilliant green. The degradation activity of *Kurthia* was studied on daily and hourly basis. Dissolved oxygen content in the control and degraded solution was estimated using electronic water analyzer. Dye-basis triphenylmethane dyes, and degradation activity of *Kurthia* was measured and the results were tabulated in Table 1(a), 1(b), 1(c). Likewise Hour-basis dye degradation activity was measured and tabulated in Table 2(a), 2(b), 2(c).

Dissolved Oxygen levels of different degraded solutions were estimated and tabulated in Table 3. Day-basis tricrylmethane dyes, and degradation activity was measured and the results were tabulated in Table 4(a), 4(b), 4(c.)

**Table 1(a)**

**Decolourization of Crystal violet by *Kurthia sp* (Day-basis)**

S.no	Concentration of Dye (ppm)	Decolourization Time (Hours)	Decolorization %
1.	10	24	100
2.	20	48	100
3.	30	72	98
4.	40	72	94

**Table 1(b)**

**Decolourization of Malachite green by *Kurthia sp* (Day-basis)**

S.no	Concentration of Dye (ppm)	Decolourization Time (Hours)	Decolorization %
1.	10	24	100
2.	20	24	100
3.	30	48	100
4.	40	48	98

**Table 1(c)**

**Decolourization of Brilliant green by *Kurthia sp* (Day-basis)**

S.no	Concentration of Dye (ppm)	Decolourization Time (Hours)	Decolorization %
1.	10	24	100
2.	20	24	100
3.	30	48	100
4.	40	48	96

**Table 2(a)**

**Decolourization of Crystal violet by *Kurthia sp* (Hour-basis)**

<b>S.no</b>	<b>Concentration of Dye (ppm)</b>	<b>Decolourization Time (Hours)</b>	<b>Decolorization %</b>
1.	1	45	100
2.	2	90	100
3.	3	135	100
4.	4	180	100
5.	5	225	98
6.	6	270	96

**Table 2(b)**

**Decolourization of Malachite green by *Kurthia sp* (Hour-basis)**

<b>S.no</b>	<b>Concentration of Dye (ppm)</b>	<b>Decolourization Time (Hours)</b>	<b>Decolorization %</b>
1.	1	45	100
2.	2	90	100
3.	3	135	100
4.	4	180	100
5.	5	225	100
6.	6	270	98

**Table 2(b)**

**Decolourization of Brilliant green by *Kurthia sp* (Hour-basis)**

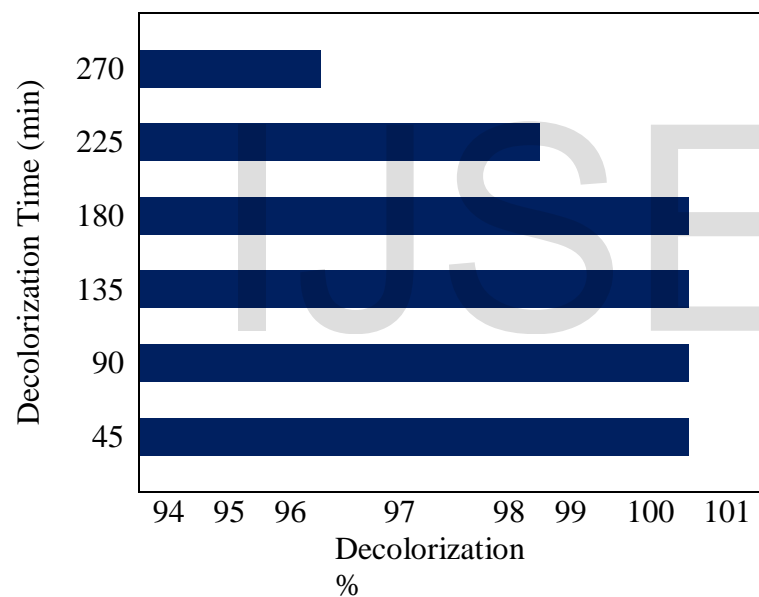
<b>S.no</b>	<b>Concentration of Dye (ppm)</b>	<b>Decolourization Time (Hours)</b>	<b>Decolorization %</b>
1.	1	45	100
2.	2	90	100
3.	3	135	100
4.	4	180	100
5.	5	225	100
6.	6	270	98

**Table 3**

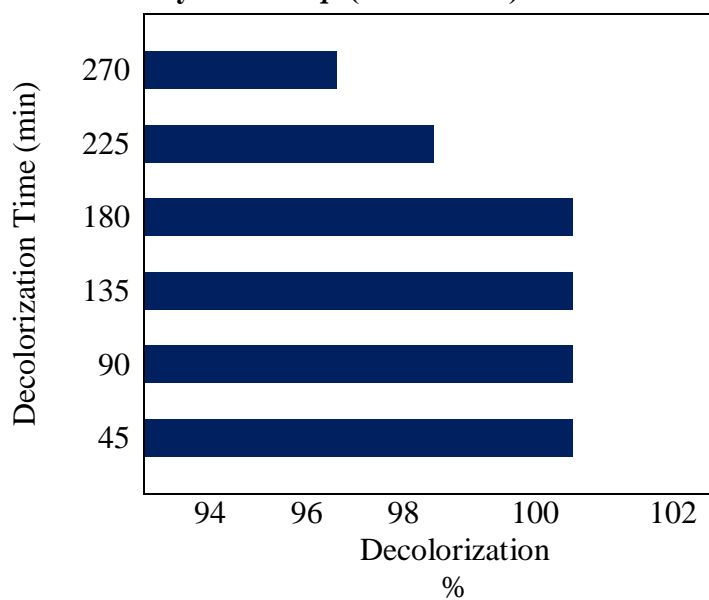
**Dissolved Oxygen Levels of Inoculated and Uninoculated Dye solutions.**

S.no	Dyes	Dissolved Oxygen (ppm)	
		Dye control	Inoculated
1.	Crysral violet	5.5	7.3
2.	Malachite green	5.6	7.3
3.	Brilliant green	5.4	7.4

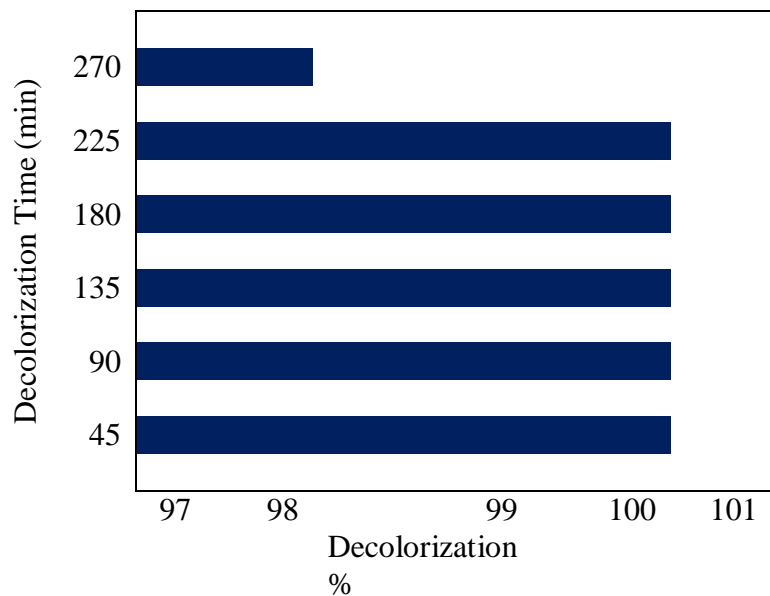
**Fig 1 Crystal Violet Decolourization by *Kurthia spp* (Hour - Basis)**



**Fig 2 Malachite Green Decolorization  
by *Kurthia sp* (Hour-basis)**



**Fig 3 Brilliant green decolorization by  
*Kurthia sp* (Hour-basis)**



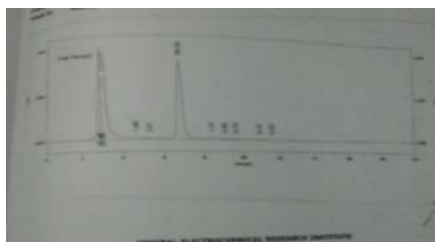
*Kurthia spp* was tentatively isolated from textile dye effluent surface colonies on yeast nutrient agar was usually rhizoid and had “medusa – head” appearance under low magnification. The isolated by microscopic, biochemical examination. Dye degradation activity was measured by using various concentrations of synthetic dye solutions prepared in our laboratory. The dyes used were crystal violet, Malachite green, Brilliant green. The degradation activity of *Kurthia* was studies on daily and hourly basis.

When decolourization was studied on day – basis, it was observed that complete decolourization occurred within 48 hours with 10ppm and 20ppm of the three dyes. 10ppm and 40 ppm of Crystal violet was degrade upto 98% and 94%, respectively in 72 hours. Similarly 30ppm of Malachite green and Brilliant green were degraded 100% within 48 hrs. 40ppm of Malachite green and Brilliant green were decolorized by 98% and 96% respectively in 48 hrs.

When decolorization was studied on hourly basis, it was observed that 100% decolourization of Crystal violet of 1ppm, 2ppm, 3ppm and 4 ppm concentration occurred with 45, 90, 135, and 180 minutes, respectively. Complete decolourization was noted in Malachite green and Brilliant green for concentration within time interval of 45 to 225 min. 6ppm of both Malachite green and Brilliant green was decolorized by 98% within 270 minutes.

The Dissolved Oxygen values of Malachite green and Crystal violet controls were 5.6ppm, 5.4ppm, 5.5ppm respectively. The Dissolved Oxygen levels of corresponding *Kurthia* treated cell free filtrate were 7.3ppm, 7.4ppm and 7.3ppm. Hence it may concluded that *Kurthia spp* it is a bacteria to depollute the effluent containing triphenylmethane dyes. It is also a non-pathogenic biological agent for decolorizing textile dye waste water containing triphenylmethane dyes.

HPLC was performed in *Kurthia* treated Triphenylmethane dyes. The HPLC results showed that chemical compound of triphenylmethane dyes were reduced and may be converted into  $\text{CO}_2$ ,  $\text{H}_2\text{O}_2$  as there were no other degraded products detected.



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