A Review on Bacterial Degradation of Triphenylmethane Dyes

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***Abstract*: Synthetic dyes persist in the environment due to their recalcitrant nature. Wide range of application of synthetic dyes in industrial and clinical set ups has led to an increased discharge of these dyes into the environment. The dyes being toxic and carcinogenic have to undergo efficient treatment before releasing into the environment. Different physical and chemical methods have been adopted for the treatment of dye effluents. However biological methods are more favoured as they are cost efficient and environment friendly. Most of the biological treatments of dyes rely on microorganisms like bacteria, fungi, algae and yeast. This review aims at providing a detailed account of bacterial degradation of widely used dyes belonging to the class of triphenylmethane dyes.**

***Keywords*: Triphenylmethane dyes, Decolourisation, Bacillus sp., Pseudomonas sp., Malachite green, Crystal violet**

I. INTRODUCTION

Synthetic dyes have a variety of applications in industrial as well as clinical set ups. They are synthetic aromatic water soluble dispersible organic colourants which interact with their substrates through physical, chemical, or mechanical attachments [1, 2]. Synthetic dyes contain an element or complex called the chromophore which is responsible for the absorption of light in a dye. Chromophores confer shading to the colour since they are equipped for absorbing light in the visible range while another complex called auxochromes gives the colour deepening when introduced to coloured molecule [3]. Based on the structure of the chromophore dyes can be categorised into different groups like azo dyes, anthraquinone dyes, nitro dyes, phthalein dyes, indigoid dyes and triphenylmethane dyes [4]. Among the different classes of dyes azo dyes, anthraquinone and triphenylmethane dyes are the most commercially used dyes [5].

A shift from natural dyes derived from plant and insect sources to synthetic dyes has been observed because of the wide range of available colours and cost efficiency of synthetic dyes [3, 6]. The application of synthetic dyes is a common practice in industries like textile, food, cosmetic, leather, paint, paper, and pulp. The textile industries have been found to be one of the major industries generating coloured effluent. Dyes do not completely bind to the substrate the loss of dye can vary from 2% for basic dye to 50% for reactive dyes [7]. Since textile industries make use of different types of dyes the effluent released is highly coloured leading to contamination of the ground and surface water in the vicinity.

Most of these synthetic dyes are recalcitrant in nature and resistant to degradation by factors like light, water, and oxidizing agents [8, 9]. The recalcitrant nature of dyes leads to the accumulation of dyes and their by-products in the environment which can be toxic or carcinogenic to living organisms [10].

Dye containing effluents have proved to be hazardous to the receiving aquatic environment as it can disturb the symbiotic ecological balance because of reduced light penetration, disturbance in the photosynthetic activity and other aquatic biological processes [1, 11]. Most of the synthetic dyes are composed of carcinogenic compounds like benzidine and other aromatic compounds [12]. Synthetic dyes are difficult to degrade in wastewater treatments because of their synthetic nature and complex aromatic structures. Due to hazardous environmental implications of synthetic dyes, effluents containing dyes should be treated for decolourization or degradation.

II. METHODS OF DYE DEGRADATION

Different degradation methods such as chemical, physical, and biological methods have been followed. The physicochemical treatments include oxidation, ozonation, electrocoagulation, adsorption, membrane filtration, flocculation, reverse osmosis, which are less effective owing to the complex molecular structure of synthetic dyes, waste produced and the cost [13, 14]. In comparison to the physicochemical methods, biological methods have been found to be advantageous due to the cost, decreased production of sludge and production of by products which are compatible with the environment [15, 16]. Biological processes degrade the complex compounds by mineralising organic contaminants without producing any toxic components [17].
In recent years, the interest in biological degradation of synthetic dyes has escalated due to the toxicity and carcinogenicity attributed with the degradation of synthetic dyes. Different microorganisms like fungi, bacteria, algae, and yeast have been found to decolourise and degrade the synthetic dyes under given environmental conditions. Reports have shown that microorganisms are able to decolourise dye-based effluents either by bio adsorption, bioaccumulation or degrade dye complexes with the help of extracellular or intracellular enzyme reduction [18].The survival and adaptability of microorganisms decides the effectiveness of the treatment [19].

III. DEGRADATION OF TRIPHENYLMETHANE DYES BY BACTERIA

Triphenylmethane dyes are brightly coloured synthetic organic dyes having molecular structure based on hydrocarbon triphenylmethane. These dyes are clearly visible due to their bright and vibrant colouration, indicating water pollution. Efficient degradation of these dyes by several bacterial species has been reported (**Table 1**).

Microbial degradation of triphenylmethane dyes by bacteria is widely reported [20, 21]. The use of broad spectrum and highly efficient dye decolourising microorganisms are essential for successful dye degradation. Factors such as dry weight of the microorganism, pH and the decolourization system affect the degradation of dyes [22]. Presently gram-negative microscopic organisms such as, *Aeromonas, Escherichia, Citrobacter, Pseudomonas, and Sphingomonas* have been found to decolorize dyes or effluents [10]. Likewise, gram positive microscopic organisms, for example, *Bacillus, Clostridium, Nocardia, Paenibacillus, Streptomyces, Micrococcus* have been found to degrade synthetic dyes [23]. There are several studies showing the degradation of dyes by single pure microorganisms as well as consortium.

Several studies focusing on biological degradation of dyes have shown that many species of Pseudomonas have the ability to decolourise triphenylmethane dyes. A study by Yatome *et al.* showed that *Pseudomonas pseudomallei* 13NA were able to degrade basic violet 3 [24]. Sarnaik and Kanekar reported the degradation of methyl violet by *Pseudomonas mendocina* MCM B-402. *Pseudomonas mendocina* also showed the ability to degrade malachite green adsorbed on chicken feathers [25]. Adsorption of malachite green on chicken feathers can hinder the degradation of chicken feathers by interfering with the metabolism of feathers by soil microorganisms [26]. Oranusi and Ogugbue explained the effect of co-substrates of the decolourisation of brilliant green and crystal violet by *Pseudomonas sp*. It was evident from this study that the percentage of decolourisation was greater in the presence of co-substrate than in the absence of co-substrate [27]. A novel species of *Pseudomonas, Pseudomonas otitidis* WL-13 isolated from activated sludge from a wastewater treatment plant of a dyeing industry showed a high capacity to decolourise TPM dyes by adsorption of the dye onto the biomass [21]. *Pseudomonas fluorescens* was also found to degrade direct orange 10228. An aerobic strain *Pseudomonas sp.* YB2 having relatively high salt tolerance and shows antibiotic resistance was identified by Tao et al for the degradation of malachite green [29].

Table 1: Bacterial biodegradation of triphenylmethane dyes

MICROORGANISMS	DYE	REFERENCE
Pseudomonas pseudomallei 13NA	Basic violet 3	[24]
Pseudomonas mendocina MCM B-402	Methyl violet	[25]
Pseudomonas mendocina	Malachite green	[26]
Pseudomonas otitidis WL-13	Crystal violet, malachite green, basic fuchsin, brilliant green	[21]
Pseudomonas fluorescens	Direct orange 102	[28]
Pseudomonas sp. YB2	Malachite green	[29]
Bacillus subtilis	Crystal violet, para rosaniline, Victoria blue	[30]
Bacillus vallismortis	Malachite green, aniline blue and brilliant green	[31]
Bacillus cereus DC11	Malachite green	[32]
Staphylococcus epidermidis	Crystal violet, Malachite green, Phenol red, Methylene red and Fuschin	[33]
Kurthia sp.	Crystal violet, malachite green, pararosaniline, ethyl violet, brilliant green and magenta	[34]
Sphingomonas paucimobilis	Malachite green	[35]
Kocurea rosea MTCC 1532	Malachite green	[36]
Enterobacter sp. CV-S1 *Enterobacter sp.* CM-S1	Malachite green	[37]
Streptomyces microflavus CKS6	Crystal violet	[41]
Streptomyces chrestomyceticus S20	Malachite green	[42]

Various species of *Bacillus* can degrade and decolorize different dyes. Yatome *et al* have reported that *Bacillus subtilis* can degrade crystal violet, para rosaniline and Victoria blue [30]. Zhang *et al* investigated the ability of spore laccase isolated from *Bacillus vallismortis* to decolourise dyes like malachite green, aniline blue, and brilliant green [31]. Deng *et al* investigated the ability of

Bacillus cereus DC11 to degrade synthetic dyes. The results showed that *Bacillus cereus* DC11 was able to decolourise TPM dyes, azo dyes and anthraquinone dyes [32].

Degradation of crystal violet, malachite green, phenol red, methylene red and fuchsin by different species of bacteria have been reported. *Staphylococcus epidermidis* degrades crystal violet, malachite green, phenol red, methylene red and fuchsin into non-toxic products33. The UV viz adsorption peaks decrease indicating the decolourisation of the dye. Hence, the appearance or disappearance of visible light absorbance peak indicates degradation.

Decolorization of crystal violet, malachite green, pararosaniline, ethyl violet, brilliant green and magenta by *Kurthia sp*. was reported by Sani and his coworkers [34]. This species exhibited only intracellular decolourising activity by removing 98% colour under aerobic conditions. The dyes such as brilliant green and pararosaniline showed 100% rate of decolourization while other dyes magenta, crystal violet and malachite green showed 92%, 96% and 96% respectively. However, ethyl violet was the least decolourised dye and severely affected cell viability. The degradation of malachite green dye by *Sphingomonas paucimobilis* under shaking conditions within 4 hours of incubation was reported by Ayed and his coworkers [35]. Malachite green has been found to be decolourised by *Kocurea rosea* MTCC 1532 under shaking anaerobic conditions [36]. Malachite green is also found to be degraded by *Enterobacter sp*. CV-S1 and Enterobacter sp. CM-S1 at concentrations up to 15mg/L at pH 6.5 [37].

Actinobacteria like *Streptomyces* have the potential for decolourising synthetic dyes majorly belonging to the azo group of dyes [38, 39, 40]. However few studies have investigated the role of Streptomyces in the decolourisation of TPM dyes. A strain of Streptomyces, Streptomyces microflavus CKS6 was found to degrade crystal violet in a two-step process of bio adsorption followed by enzymatic action with lignin peroxidase having a prominent role in degradation [41]. *Streptomyces chrestomyceticus* S20 showed capability of degrading malachite green. Addition of carbon and nitrogen sources like 1% glucose and yeast extract greatly enhanced degradation [42]. In another study it was observed that parameters such as pH, dye concentration, agitation speed, biomass and oxygen greatly influenced the decolourising ability of *Streptomyces sp.* In a comparison between the decolourisation ability of live and dead cell it was found that live showed higher efficiency at an optimum pH of 7 [43].

IV CONCLUSION

The toxicity associated with synthetic dyes and their by-products calls for urgent attention toward the treatment of these compounds. Biological methods have been found to be effective in comparison to the physical and chemical methods because of the non-toxic end product, cost effectiveness and increased efficiency of biological methods. The use of bacteria as single species or in consortium with other species has been found to be effective in the treatment of many triphenylmethane dyes. Many studies have found bacterial consortium to be more effective when compared to a single species. Similarly studies have also shown that facultative anaerobes have an increased decolourisation potential. Degradation of dyes using bacteria is being largely followed and considered important in effluent treatments. Certain strains of bacteria have the ability to completely decolourize and break the dyes into simpler non-toxic products. They are comparatively easier to culture given the appropriate conditions when compared to fungi and grow in a faster rate. Genetic manipulations can be done in bacterial strains to improve their efficiency. Decolourisation ability of live and dead bacterial cells have also been investigated which shows that live cells are efficient in degrading and decolourising dyes when compared with dead cells. Bacteria are able to degrade dyes due to their enzymatic machinery, where the enzymes can be intracellular or extracellular. Mainly the enzymes involved in the degradation process belong to the oxidoreductase class of enzymes. These characteristics make them the most preferred organisms in the degradation. The commonly used bacterial genus to decolourize TPM dyes are *Pseudomonas, Citrobacter, Desulfovibrio, Mycobacterium*. Degradation or decolourisation efficiency is also affected by other operational parameters like pH, temperature, dye concentration, biomass, carbon and nitrogen availability. Hence optimization of these parameters is required for obtaining maximum degradation of synthetic dyes.

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