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Review Article:

**BIOREMEDIATION OF DISPERSED DYES BY BACTERIAL ISOLATES**

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GUJARAT UNIVERSITY, AHMEDABAD, GUJARAT – 380 009.[nkbmutual@gmail.com](mailto:nkbmutual@gmail.com) and [husolanki@yahoo.com](mailto:husolanki@yahoo.com)**ABSTRACT:**

The requirement of present day society of chemical technology pertaining to clothing is almost beyond comprehension. Systematic dyes have a wide range of application in textile, paper and leather industry. Wastewater from such textile industry is recalcitrant containing coloured dyes and toxic materials. The remediation of coloured effluents in wastewater treatment system is mainly physical chemical procedures, but it leads to generation of highly hazardous sludge. Natural microbes to degrade dye have long been known for treating textile effluents. Several bacterial isolates are screened from effluent sample of textile industry capable of decolorizing dispersed dye, Basic violet 14, Basic violet 3, acid blue 90 Brown S3BL and Yellow Brown-2RC which are mostly used in synthetic dyeing. In presence of salt medium, 0.05 gm % dye is decolorized in acidic condition at room temperature in 24 hours. Various parameters influence efficient decolorization of dyes by bacterial isolates have been studied and promising results are reported.

**KEY WORDS:** *Polycyclic hydrocarbons, bacterial isolates, synthetic dyes.*

**INTRODUCTION:**

Globally, in recent days, dye wastewater has become one of the main sources of severe pollution problems because of the higher demand for textile products and the proportional augmentation in production and applications of synthetic dyes (Santos *et al.* 2007 and Carliell 1998). An educated guess has been made that over 10,000 diverse dyes and pigments are used industrially and more than 0.7 million tons of dyes are produced annually over the world. In textile industry, up to 200,000 tons of these dyes are lost to effluents every year during dyeing and finishing operations as a result of inefficiency in the dyeing process (Zollinger, 1987 and Ji *et al.* 2008). For this reason economical and bio-friendly approaches are needed to remediate dye-contaminated wastewater from various industries. Till date various bacterial strains have been tested for their efficacy of decolorizing textile dyes. Among several diverse groups of synthetic dyes, triarylmethane (also

called triphenylmethane) dyes are one of the most commonly used in the textile industries. Their usage constitutes about 30%–40% of the total consumption of dyes (Gregory, 1993), and they are applied extensively on nylon, cotton, wool, and silk. They are also used for colouring food, oils, fats, waxes, varnishes, cosmetics, paper, leather, and plastics (Gupta *et al.* 2003) as well as for staining specimens in bacteriological and histopathological processes. With dye tinctorial value usually high, less than 1 ppm of dye in water produces obvious coloration, and the extensive use of these dyes have resulted in highly colored effluents that may affect gas solubility in water bodies and significantly decrease photosynthetic activity in aquatic life because of reduced light penetration. In addition to this triarylmethane dyes are mostly known for its toxic and carcinogenic characteristics. Many reports have documented this fact that textile dyes and effluents have toxic effects on plants which execute imperative ecological functions such as providing a habitat for wildlife, protecting soil from erosion, and providing the organic matter that is so significant to soil fertility. As a result, it is important to develop well-organized treatment strategies for removal of colour from dye wastewater.

Bioremediation utilizes the metabolic versatility of microorganisms to degrade hazardous pollutants. For present study goal of bioremediation is to transform organic pollutants into harmless metabolites or mineralize the pollutants into carbon dioxide and water. Many factors influence microorganisms to use pollutants as substrates or co-metabolize them. Therefore, understanding catabolic pathways and mechanisms and responsible enzymes is an effective means to define important factors for efficient cleanup of pollutants. Decolourization by biological means may take place in two ways: either by adsorption (or biosorption) on the microbial biomass or biodegradation by the cells. Biosorption involves the entrapment of dyes in the matrix of the adsorbent (microbial biomass) without destruction of the pollutant, whereas in biodegradation, the original dye structure is fragmented into smaller compounds resulting in the decolourization of synthetic dyes. Biodegradation is a very broad field and involves uses of a wide range of microorganisms to break chemical bonds. This review is focused on bacterial catabolic pathways of selected aromatic pollutants *viz.* PAHs naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene, the heterocycles dibenzofuran, carbazole, dibenzothiophene, and dibenzodioxin, and alkylated PAHs. For convenience, the chlorinated hydrocarbons degraded by microorganisms are divided in to three classes: aliphatic, polycyclic and aromatic.

Table 1: Biodegradation of chlorinated aliphatic compounds

Compound	Microorganism	Plasmid*
2-monochloropropionic aci	<i>Pseudomonas sp.</i>	-
2-Monochloroacetic acid	<i>Alcaligenes sp.</i>	pUU204 (53kb)
1,2-dichloroethane	Anaerobes	-
	<i>Xanthobacter autotrophicus</i> (GJ10)	-
	Methane-utilising bacteria	-
	<i>P. fluorescens</i>	-
1,1,1-TCE	Anaerobes	-
	Methanotrophs	-
Perchloroethane	Methanosarcina sp.	-
1-Chlorobutane	Aerobes and anaerobes	-
1,2-Dichloropropane	<i>P. fluorescens</i>	-
1,3- Dichloropropane	Aerobes	-
1,9-Dichlorononane	Anaerobes	-
TCE	Anaerobes	-
	Anaerobes	-
	Aerobes	-
	Aerobes	-
	Methane-utilising bacteria	-
	<i>P. putida</i>	-
	Methane-oxidizing bacteria	-
	<i>P. fluorescens</i>	-
3-Chloro-4-hydroxybenzaldehyde	Anaerobes	-
Chlorolignin	<i>Sporotrichum pulverulentum</i>	-
Monochloro-, bromo- iodoalkanes	<i>Arthrobacter sp.</i>	-

Source: Biodegradation of Halogenated Organic Compounds G. RASUL CHAUDHRY\* AND S. CHAPALAMADUGU

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Halogenated aliphatic compounds are prevalent groundwater contaminants and are significant components of hazardous wastes and landfill leachates. Many hazardous halogenated aliphatic compounds released from industrial, commercial, and agricultural sources are chlorinated or brominated alkanes and alkenes that contain one to three carbon atoms, such as halogenated

alkanoic acids (HAA), haloalkanes, TCE, trichloroethane (TCA), and ethylene dibromide (EDB). Chlorinated ethanes and ethers have been commonly used as refrigerants in manufacturing, as solvents in the dry-cleaning (metal and plastic) and lacquer industries, and in semiconductor manufacturing. Their apparent hazard to human health has prompted investigations concerning their fate in subsurface waters and in the soil environment. The transformations of some chlorinated aliphatic compounds by soil and aquatic microorganisms are listed in Table 1.

Although there are very few reports available on biodegradation and detoxification of textile dyes containing triarylmethane dyes, a detailed research has been carried out by Ogugbue and Thomas 2011. This research, documents the fact that strain of *Aeromonas hydrophila* is capable of efficiently degrading triarylmethane dyes. According to him degradation of the dyes by the bacterial isolates was found to be dependent on concentration of dyes, pH, aeration as well as temperature and presence of co-substrate. Phytotoxicity tests carried out on *Triticum aestivum*, *Hordeum vulgare* and *Lens esculenta* also indicated detoxification of the dyes after degradation as decolorized samples exhibited lower toxic effects than the raw dyes.

Details of three dyes used by Ogugbue and Thomas, 2011 in this research are given below;

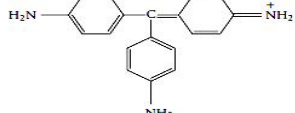
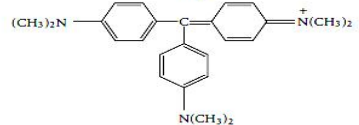
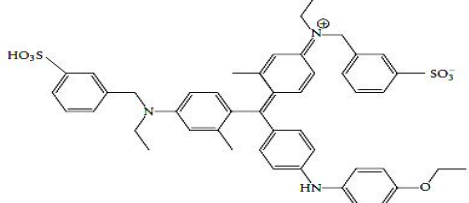
Name	Color index no.	Structure	Molecular weight	$\lambda_{max}$ (nm)
Basic violet 14	42510		337.85	546
Basic violet 3	42555		408.0	593
Acid blue 90	42655		854.02	554

Figure: 1

### Degradation of halogenated compounds:

Xenobiotic compounds have been utilized comprehensively in crop growing as herbicides and insecticides and in the manufacturing industry as solvents and degreasers. Communal apprehension with reference to probable perilous effects of these chemicals on human beings and their surroundings has focused mainly on a small number of classes of compounds. Of these compounds, chlorinated hydrocarbons are the most publicized. Most of the chlorinated compounds are highly toxic, and because they are often insoluble, they escape degradation. On the other hand,

the microorganisms exposed to these synthetic chemicals have evolved the ability to utilize some of them. Bacteria of several diverse genera have been shown to degrade chlorinated hydrocarbons. Most of the xenobiotic degrading microorganisms harbour plasmids which code for the catabolic genes. By understanding the biochemistry and genetics of plasmid-borne degradation and by using the recombinant DNA techniques, it is possible to typify the suitable genes and transfer them to construct improved strains with enhanced ability for degradation of several toxic compounds. One of the objectives of genetic engineering of toxic chemical-degrading microorganisms is to develop so-called "superbugs," capable of detoxifying or decontaminating the toxic chemicals in the natural environment. To establish the potential applications of the recombinant strains in the surroundings, the strains must be stable members of the indigenous micro-flora and the recruitment of catabolic enzymes and gene regulators with appropriate effectors specificities (by natural gene transfer or by laboratory manipulation) to create new hybrid pathways for chlorinated compounds must not significantly alter the host or the natural ecosystem. Even though the risk of releasing recombinant organisms is not known, the prospects for the construction of catabolic pathways to effect mineralization and detoxification of halogenated compounds are encouraging. However, the utility of constructed organisms in dealing with problems related to environmental pollution in nature has yet to be tested. Obviously, the most effectual means of avoiding further pollution lies in restricting the use of recalcitrant compounds and replacing them with non-recalcitrant alternatives, as well as reducing and decontaminating the toxic waste at the site of generation. Genetically engineered microorganisms could be useful in decontaminating waste in contained environmental situations such as biodegradations reactors, dump sites, and waterworks systems.

Another such research carried by Abadulla *et.al.* (2000) showed that *Trametes hirsuta* and a purified laccase from this organism were able to degrade triarylmethane, indigoid, azo and anthraquinonic dyes. According to Bollag and Leonowicz (1984) and Bortone (1995) laccases can be used for the treatment of effluents from pulp mills or from other industries containing chlorolignins or phenolic compounds. Call and Mucke (1996) have reported that, several processes using laccases as well as immobilized laccases have been developed for the treatment of phenolic effluents and polycyclic aromatic hydrocarbons. Diagrammatic representation of textile dyes used in above mentioned research is as under;

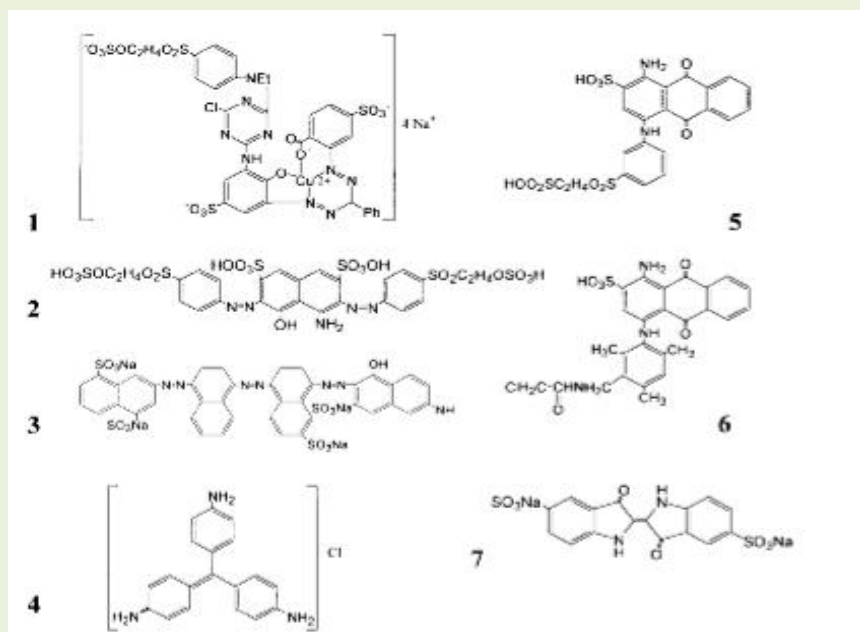


Figure: 2

Source: Elias Abadulla et.al. 2000, Department of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria Decolorization and Detoxification of Textile Dyes with a Laccase from *Trametes hirsuta*

Dye	Structure no. <sup>a</sup>
Reactive Blue 221	1
Reactive Black 5	2
Direct Blue 71	3
Basic Red 9 Base	4
Reactive Blue 19	5
Acid Blue 225	6
Acid Blue 74	7

Name of the dye and structure number corresponds to the Figure: 2

Here, Decolourization of dyes by *T. hirsuta* was mainly ascribed to extracellular laccase activity, further more Electron-donating methyl and methoxy substituent seemed to enhance laccase activity, while electron withdrawing chloro, fluoro, and nitro substituent inhibited oxidation of azophenols and other substituted phenols and phenol analogs by fungal laccases. According to O'Neill et.al. 2000 respiration inhibition test proves that anaerobic degradation of azodyes rendered effluents more toxic by generating amines, while a second aerobic treatment eliminated this toxicity. Chivukula and Renganathan (1995) have suggested reaction mechanism for degradation of azo dyes by laccases involving the conversion of azo-nitrogen in to molecular

nitrogen. In conclusion it could be said that both water consumption and effluent toxicity in textile dyeing could be reduced by “enzyme remediation” with laccases.

### **Conclusion and future recommendations:**

In addition to this, Proteomics and metabolomics have been recently employed in studies of environmental microbiology and have shown their high impact on the field of biodegradation and bioremediation. Proteomics is an effective technique to identify proteins and their functions involved in the biodegradation of aromatics while metabolomics can be used to profile degradation products of PAHs and primary metabolites in response to PAH exposures. A good number of genomic sequences or expressed sequence tags (ESTs) of bacteria are presently accessible. These comprise quite a few PAH degrading bacteria in the genus of *Mycobacterium*, *Acinetobacter*, *Arthrobacter*, and *Burkholderia*.

A great deal of work remains to be done in carrying out field studies based on laboratory-scale experiments prior to commercially feasible systems are made accessible using plant-associated endophytic and rhizospheric bacteria to degrade a extensive array of toxic organic compounds of concern in environmental soil. Plant-associated endophytes may offer more potential for bioremediation than plant-associated rhizospheric bacteria since: (i) the use of endophytes that are inhabitant to the host plant reduces competition between bacterial strains and may eradicate the need for re-inoculation, (ii) toxic organic contaminants can remain in the plant xylem for up to two days facilitating their degradation by endophytes, and (iii) endophytes can be isolated from host plants of interest and genetically enhanced with genes encoding degradation enzymes of interest before re-inoculation for bioremediation. Nevertheless, with a international political shift towards sustainable and green bioremediation technologies, the use of plant-associated bacteria to degrade toxic synthetic organic compounds in environmental soil may provide an resourceful, financially viable, and sustainable green remediation technology for our twenty first century environment.

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