

# Degradation of Industrial Fabric Dyes used in Sanganer Area by Endophytic Microbes

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## ABSTRACT

Dyes and dyestuffs find use in a wide range of industries but are of primary importance to textile manufacturing. Wastewater from the textile industry can contain a variety of polluting substances including dyes. Increasingly, environmental legislation is being imposed to control the release of dyes, in particular fabric-based compounds, into the environment. The ability of microorganisms to decolourise and metabolise dyes has long been known, and the use of bioremediation based technologies for treating textile wastewater has attracted interest. Within this review, we investigate the mechanisms by which diverse categories of microorganisms, such as the white-rot fungi and anaerobic bacterial consortia, bring about the degradation of dyestuffs. Rapid industrialization has given rise to various unwanted elements that accumulated in the biosphere up to toxic levels to degrade the natural environment. Scientific developments are considered as key factors for progress of both developing and under developed countries, but unfortunately, most of the industries in these countries do not have proper waste treatment facilities and releasing a large quantity of effluents. A majority of xenobiotics (either untreated or partially treated) released from industries are mixed up with the natural water bodies and to the soil of the biosphere. Untreated or partially treated textile effluents are highly toxic, as they contain a large number of toxic chemicals and heavy metals. The problem of water pollution due to the discharge of industrial wastewater into natural water bodies was witnessed by western countries in 19th century and also in India after independence

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## EXPERIMENTAL STUDIES DONE BEFORE:

FOR EXAMPLE:-In a particular study, based on colony morphology characteristics, a total of 19 fungal endophytes were isolated from root of *Calotropis Procera* a traditional Indian medicinal plant. All fungal isolates were screened for their dye degradation ability. The dyes used as test dyes were Rose Bengal (RB), fabric dye Methyl Red (MR), Coomassie Brilliant Blue (CBB) and Methylene Blue (MB) and the concentration of each dye in the experiment was kept 100mg/L. Among the 19 fungal endophytic isolates (CPR1-CPR19), only one isolate CPR4 showed strong dye decolourization capability against all the four test dye. Dye decolourization ability by the isolate CPR4 was determined to be 97.4%, 87%, 65% and 45% for Rose Bengal (RB), Methyl Red (MR), Coomassie Brilliant Blue (CBB) and Methylene Blue (MB) respectively. Complete colour decolourization was observed with rose Bengal followed by Methyl Red. Glucose minimal medium was used for liquid and solid culture of fungal isolates. Fungal biomass production in the presence of four test dye was studied and compare with control culture of fungal endophytes. Effect of temperature, pH, stationary and agitation conditions on dye degradation was also studied

## INTRODUCTION

The term Endophyte refers to the organisms which throughout or part of its life cycle invade the tissues of living plants and cause a symptomatic infections. Endophytic

organisms have received considerable attention after they were found to protect their host against insect, pest, pathogens and even domestic herbivorous (Webber, 1981). Almost all the plant species (-400,000) are harbor one or more endophytic organisms (Tan and Zou, 2001). Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). Leaves, roots, and stems part of one adult plant with healthy appearances were collected from the site area. After the disinfection, fragments of each plant parts were homogenized in 5 ml of sterile distilled water with a blender and prepared three serial dilutions of each sample. 50µl of each dilution of a sample was spreaded onto the media plates of Potato Dextrose Agar for isolation of fungi. The isolation of pure fungal culture can be achieved by single spore culture method. In this technique a previously diluted mixture of fungal spores is spread onto the surface of agar medium. Once discrete, well separated fungal colonies are obtained, then each may be picked up with a sterile needle and transferred to fresh Potato dextrose agar slant. These slants can be preserved as the pure or stock cultures. Microbial decolourisation and degradation is an environmentally friendly and cost competitive alternative to chemical decomposition processes (Verma and Madamwar, 2003)[1]

Among endophytic microbes Endophytic fungi inhabit plant tissues, in either a symbiotic or mutualistic relationship, without harming the host plant. They are known for the production of secondary metabolites, which shield the host from invading pathogens. Endophytic fungi produce extracellular enzymes like laccases that have a potential role to play in dye decolorization. Dyes are complex organic compounds that are derived from biological, chemical, and physical processes and are useful for all industries, but mainly the textile, leather, paper, and food industries. In contrast, the world faces ecological problems due to the toxicity of synthetic compounds. They are nondegradable and persist for a long time. This chapter focuses on the decolorization of various dyes through endophytic fungi using various processes like biomagnification, biosorption, bioaccumulation, and enzymatic degradation. Moreover, this chapter explains the efficiency of endophytic fungi in the degradation of various dyes, for example, Congo red, methyl orange, methyl red, and crystal violet. Therefore, it is essential to carry out toxicity studies on dye degradation and to develop an eco-friendly technology that may degrade dyes easily.

Also, endophytic bacteria were isolated from the roots and shoots of three wetland plants, *Typha domingensis*, *Pistia stratiotes* & *Eichhornia crassipes*, and identified by 16S rRNA gene sequencing. Textile effluent-degrading and plant growth-promoting activities of these endophytes were determined. The analysis of endophytic bacterial communities indicated that plant species had a pronounced effect on endophytic bacterial association and maximum endophytes (56.5%) were associated with *T. domingensis*. These endophytic bacteria mainly belonged to different species of the genera *Bacillus* (39%), *Microbacterium* (12%) and *Halomonas* (12%). Eight of the 41 strains showing maximum efficiency of textile effluent degradation also exhibited plant growth-promoting activities such as production of indole-3-acetic acid and siderophore, presence of 1-amino-cyclopropane-1-carboxylic acid deaminase, and solubilization of inorganic phosphorous. This is the first study describing the diversity and plant-beneficial characteristics of the textile effluent-degrading endophytic bacteria associated with wetland plants. *T. domingensis* showed better growth in textile effluent and also hosted maximum number of endophytic bacteria in roots and shoots. The interactions between *T. domingensis* and its associated endophytic bacteria could be exploited to enhance the efficiency of constructed wetlands during the remediation of industrial effluent.[2]

It has been found that, it was found that the endophytic fungal isolates performed better in decolorizing targeted dyes than bacterial isolates. A previous study reported that fungi have more tolerant to high concentrations of polluting chemicals than bacteria (Casieri et al., 2008). Fungi produce various extracellular enzymes, resulting in enhanced bioremediation rates for most of the pollutants (Kaushik and Malik, 2009). Further, fungi have a greater physical contact with the environment due to the presence of increased cell-surface ratio. Therefore, fungal systems appear to be most appropriate in the treatment of textile dyes (Ezeronye and Okerentugba, 1999) and also, they had shown better dye reduction potential over the bacteria (Fu and Viraraghavan, 2002). It has been reported that the white rot fungus *Phanerochaete chrysosporium*, grown under ligninolytic

conditions, had shown to metabolize crystal violet by sequential N demethylation of the parent compound, which had catalyzed by lignin peroxidase (Cha et al., 2001). In a previous report it was demonstrated that supernatants from *Fomes sclerodermeus* with laccase activity were able to degrade malachite green dye (Papinutti et al., 2006). Decolorization studies with *Trichoderma virens* showed high decolorization ability (99.6%) for brilliant blue dye (Sweety et al., 2017). The current study also showed the ability of the reduction of both dyes by the bacterial isolates. The reason for effective and faster decolorization of the textile dye by bacteria might be associated with the metabolic activities and interactions of the strains (McMullan et al., 2001; Phugare et al., 2011). It has been reported in a previous study that the significant ability of dye reduction by bacterial species, *Bacillus* sp. and *Pseudomonas* sp. isolated from textile dye effluent contaminated soil (Sriram et al., 2013). Further, the efficacy of *Bacillus subtilis* in decolorization of fabric dyes have been previously reported (Cheria et al., 2012; Ali et al., 2014). The current study clearly showed that biofilms has high decolorization ability than their monocultures. It has been reported that different microbial species present in consortia of biofilms each with different metabolic degradation pathway are capable of degrading several pollutants including dyes and heavy metals either individually or collectively (Gieg et al., 2014; Mitra and Mukhopadhyay, 2015). Decolorization of synthetic dyes using consortia offers advantages over the use of single microbial strains (Sudha et al., 2014) due to higher degrees of biodegradation resulted from synergistic metabolic activities of the microbial community (Allam, 2017). It has been reported that mixed bacterial cultures from different habitats showed high decolorization of dye. Molecules in 15 days (Knapp & Newby, 1995). Similar results have been reported by another study that immobilized bacterial consortium of the three bacterial species (*Sphingomonas paucimobilis*, *Rhizobium radiobacter*, and *Bacillus subtilis*) had the ability to decolorize fabric dyes more efficient than free bacterial cells of single culture (Allam, 2017). Mahmood et al. (2015) found that, the consortium of 6 bacterial isolates was able to decolorize 84% of 200 ppm of red, green, black, yellow, and mixed dyes within 24 hours while individual strain required 72 hours.[3]

## DISCUSSION

Limited options exist for efficiently and effectively treating water runoff from agricultural fields and landfills. Traditional treatments include excavation, transport to landfills, incineration, stabilization, and vitrification. In general, treatment options relying on biological methods such as bioremediation have the ability to be applied in situ and offer a sustainable remedial option with a lower environmental impact and reduced long-term operating expenses. These methods are generally considered ecologically friendly, particularly when compared to traditional physicochemical cleanup options. Phytoremediation, which relies on plants to take up and/or transform the contaminant of interest, is another alternative treatment method which has been developed. However, phytoremediation is not widely used, largely due to its low treatment efficiency. Endophytic phytoaugmentation is a variation on phytoremediation that relies on augmenting the phytoremediating plants with exogenous strains to stimulate associated plant-microbe interactions to facilitate and improve remediation efficiency. In this review, we offer a

summary of the current knowledge as well as developments in endophytic phytoaugmentation and present some potential future applications for this technology. There has been a limited number of published endophytic phytoaugmentation case studies and much remains to be done to transition lab-scale results to field applications. Future research needs include large-scale endophytic phytoaugmentation experiments as well as the development of more exhaustive tools for monitoring plant-microbe-pollutant interactions.[4]

Pollutants associated with industrial and agricultural runoffs are of concern to human and ecological health as they can be challenging to efficiently and effectively treat. For example, wood treatment and petroleum wastes can contain high levels of carcinogenic polycyclic aromatic hydrocarbons (PAHs); livestock wastes contain high levels of nitrates associated with “baby blue syndrome” and algal blooms; and urban runoffs contain heavy metals such as zinc and lead that are known to be toxic to humans and animals.<sup>1-4</sup> Plant-based remediation, or phytoremediation, directly uses plants and their associated microbes in situ for the stabilization or reduction of contaminants. Phytoremediation has been used for remediation in soil, sludge, sediment, surface water, and groundwater for a diverse range of contaminants.<sup>5-7</sup> This wide remedial ability is owed in part to the multifarious levels of contaminant treatment. Plants have the ability to control, degrade, or remove contaminants. Below-ground techniques rely on transformation, stabilization, or degradation stimulation. Once absorbed into the plant, transformation, stabilization or immobilization, and degradation can also occur. Methods of phytoremediation are thoroughly reviewed in Salt et al. and Ali et al

Phytoremediation has been used to remediate numerous chemicals including metals, radionuclides, pesticides and herbicides, excessive nutrients, and organic pollutants.<sup>1-2</sup> Depending on the location and desired treatment outcome, there are several types of phytoremediation planting schemes and applications that have been shown to be successful. The most common phytoremediation applications are riparian buffer strips, which consist of a strip of plantings along a wetland, stream, river, or lake, or a vegetation filter, which is used more commonly for managing municipal wastes and landfill leachates.[5]

Along with phytoremediation, in situ bioremediation is another in situ treatment option that is more ecologically friendly than traditional remediation technologies.<sup>15</sup> Bioaugmentation is a common bioremediation strategy that consists of adding exogenous microorganisms such as endophytes to remediate contaminated sediments and soils. However, in this context, bioremediation may be ineffective or inefficient if the bioaugmented strain is unable to thrive under the specific physical site conditions and local microbial ecology. In endophytic phytoremediation, endophytes interact and exchange genes with both the rhizospheric and phyllospheric bacterial communities.<sup>7</sup> In doing so, the overall microbial community develops degradation capacities without requiring survival of the donor strain. Thus, the combined use of endophytic augmentation and phytoremediation, or endophytic phytoaugmentation, may offer an effective option for in situ treatment of runoff and waste systems.

Endophytic phytoaugmentation is a promising area of research, with numerous direct and indirect benefits. For instance, endophytes are known to help the growth and health of various bioenergy- and biofiber-related crops, including poplars, willows, and cotton.<sup>6</sup> Primary and secondary wood products from poplar and willow trees, including pulp and paper, lumber, veneer and plywood, composite panels, structural composite lumber and pallets, furniture, containers and utensils, and animal feed, are expected to increase.<sup>2</sup> Furthermore, phytosystems also help aid in carbon dioxide (CO<sub>2</sub>) sequestration and may be useful for reducing greenhouse gases.<sup>22</sup> Endophytic phytoaugmentation may also be useful for agricultural systems for applications such as healthier tomato crops or grapevine growth.<sup>1</sup> This approach may also be used for biocontrol systems to provide enhanced aesthetics, to act as soil stabilizers, and to reduce dust dispersal.<sup>6</sup>

Plants are colonized by a range of microflora such as bacteria, fungi, yeasts, viruses, and protists, as well as epiphytes including algae and nematodes. Plant-microbe populations are dynamic, and variations in microbial communities that are influenced by the large fluctuations in the physical and nutritional conditions, as well as other biotic and abiotic influences, have been observed.<sup>2</sup> Some microorganisms, predominantly bacteria and fungi, are recruited to enter the plant locally and systemically as endophytes, establishing asymptomatic or mutualistic relationships. Endophytes are found systemically in roots, stems, leaves, seeds, fruits, tubers, ovules, and some nodules.<sup>2</sup> Endophytes may be recruited to their host through chemotaxis, electrotaxis, or simply accidental encounter and are most commonly recruited from the roots.<sup>1</sup> Roots have been shown to have the highest localized concentration of endophytes, and endophytic densities tend to decrease from stem to leaf.<sup>39,40</sup> The most commonly reported endophytic locations are the intercellular spaces and xylem vessels.<sup>41</sup> Endophytic communities depend on the taxa within a given community, host genotype and corresponding host developmental stage, inoculum density, temporal and seasonal conditions, plant location, and environmental conditions.<sup>1</sup> Though dynamic, many endophytic communities have been shown to contain common soil taxa such as *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Fabricspirillum* [6]

### Diversity and Function of Endophytes

Endophytes have been isolated from a diversity of plants, yet their exact function and associations remain unclear.<sup>3</sup> For instance, it is unclear how endophytes interact with each other in the plant, and little is known about the complex endophyte-host molecular interactions or their gene regulation and expression.<sup>2</sup> Endophytes seem to form diverse and complex associations with their hosts, including mutualistic and symbiotic relationships.<sup>5</sup> In many cases, endophytes are believed to be beneficial to their plant hosts through nitrogen fixation, accelerated seedling emergence, protection from environmental stressors, enhanced nutrient availability and vitamin supply, and contaminant protection and removal[6]. Endophytes are capable of producing bioactive compounds associated with increased plant growth and health, and offer protection from abiotic and biotic stresses.<sup>7</sup> Endophytes may also offer their plant host protection and defense against microbial diseases, insects, and nematodes.<sup>17</sup> For example, endophytic actinobacteria



offer defense against the pathogenic fungus *Gaeumannomyces graminis* in wheat and potatoes, and the endophyte *Curtobacterium flaccumfaciens* protects citrus plants from the pathogen *Xylella fastidiosa*. Interestingly, even some mycorrhizal fungi themselves have endosymbiotic bacteria for protection.<sup>5</sup> More in-depth endophyte reviews are provided by Newman and Reynolds, Sturz et al., Strobel and Daisy, Mercado-Blanco, Tadych and White, and Lodewyckx et al. [8]

### Endophytic Augmentation

Many endophytes have shown a natural capacity for xenobiotic degradation.<sup>9</sup> Further, plant-associated microorganisms capable of direct degradation are more abundant among endophytes than in the rhizosphere at contaminated sites. This may be because the plants themselves selectively enrich degraders inside and around the phytoremediating host plants. Siciliano et al. found that plants grown in soil contaminated with xenobiotics naturally recruit endophytes with contaminant-degrading genes. This selective enrichment suggests a potential against a wide-range of contaminants and sites. The natural ability of some endophytes to degrade xenobiotics has been investigated with regard to improving phytoremediation efficiency

Endophytic phytoaugmentation with naturally occurring xenobiotic-degrading endophytes have the advantage of reduced competition in the internal plant tissue and do not require re-inoculation, but there is still a need to determine what conditions help support a successful augmentation event. Additionally, natural endophytes have the potential to be isolated and genetically enhanced to degrade target compounds once reintroduced to the host, though the ecological implications of genetically altered microbes will need to be fully characterized.

A more in-depth understanding of the three-way plant-microbe-contaminant interactions is needed to capitalize on the potential benefits. In the future, tools will be needed to determine three-way interactions before, during, and after endophytic phytoaugmentation. Though green fluorescent protein tagging has been effective in monitoring endophyte-associated HGT at small scale (as previously discussed), more environmentally relevant, exhaustive, and field-level techniques and tools are needed. To do this, improved metagenomic, metatranscriptomic, and metaproteomic work on plant-microbe relationships is needed to fully understand and thereby optimize the augmented phytoremediation system. As methods of genomic and proteomic analysis become cheaper and faster, it has become more feasible to determine the relationship between the biotic systems and the pollutant systems. For example, targeting pollutant catabolic genes within endophyte communities using quantitative gene expression may be a useful tool for assessing colonization and remediation. Other protein-associated techniques, such as modified enzyme-linked immunosorbent assay (ELISA test) and chromatin immunoprecipitation sequencing (ChIP-Seq), may help accurately describe the DNA-protein interactions between the plant and microbial community. Given the large numbers of environmental variables and parameters, these tools need to be applied to more field-scale endophytic phytoaugmentation studies to fully characterize the remediation potential. With a more complete understanding of the “-omics” associated with remediation and of the

changes in field-level parameters, a system for reproducible and reliable endophytic phytoaugmentation may be established. Simple biomarkers or indicators of remediation (e.g., monitoring levels of a specific gene or protein) along with more exhaustive tools and techniques to monitor colonization and communications, would be valuable for large-scale or long-term projects. Though many research gaps remain, the use of endophytic phytoaugmentation may provide an economical and environmentally friendly alternative to traditional remediation techniques.

### CONCLUSION

Fabric dyes are known as industrially synthesized organic compounds, and these fabric dyes are identified by their fabric bonds (N=N). Mixtures of these synthetic dyes which are unbound to the fiber get released into the environment and that will ultimately lead to bioaccumulation. Bioaccumulation of these dyes constitutes a serious environmental hazard. Several physicochemical methods have been applied to the treatment of textile wastewater, but these methods have many limitations due to high cost, low efficiency, and secondary pollution problems. As an alternative to physicochemical methods, biological methods comprise bacteria, fungi, yeast, algae, and plants and their enzymes which received increasing interest due to their cost-effectiveness and eco-friendly nature.

Decolorization of toxic fabric dyes by biological processes may take place either by biodegradation or biosorption. A variety of oxidative and reductive microbial enzymes may also be involved in the degradation of dyes. Fabric reductase, peroxidase, laccase, and other important enzymes synthesized by these microbes have shown 80–90% efficacy in decolorizing the textile dyes. Green synthesis of nanoparticles and their mediated fabric dye degradation are the latest and effective methods used for treatment of hazardous effluent samples. Toxicity evaluation of pure dyes and degraded dye product using phytotoxicity and biotoxicity study is given a clear chart of the most effective methods. This review provides an overview of decolorization and degradation of fabric dyes by biological processes and establishes the fact that these Microbes and enzymes are significantly effective biological weapons against the toxic fabric dyes.[8]

In an example *Staphylococcus epidermidis* MTCC 10623 was examined for its potential to decolorize Basic Red 46 dye (BR 46), an extensively used dye in textile industry. *S. epidermidis* showed 99.6% decolorization of BR 46 at pH 9.0 and 40°C temp. after 6 h incubation. Addition of glucose (0.3%) and ammonium sulfate (0.1%) as carbon and nitrogen source enhanced the decolorization ability. Decolorization of BR 46 with bacterial cells immobilized over polyurethane foam (PUF) and nylon mesh (NM), resulted in 99.7 and 99.6% decolorization after 6 h incubation. UV-Vis and FTIR analysis of metabolites formed after bacterial treatment suggested that decolorization was due to degradation and not owing to adsorption. Phytotoxicity assay of these metabolites had no adverse effects on germination rate of test plants, which revealed that the treated effluent was safe for irrigation.[7]

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