A REVIEW ON BIOREMEDIATION OF AZODYES USING MICROBIAL CONSORTIUM FROM DIFFERENT SOURCES

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Abstract – The widespread industrialization and unconstrained growth of modern textile production facilities coupled with the lack of proper treatment facilities have proliferated the discharge of textile dye effluents enriched with toxic, recalcitrant, carcinogenic and mutagenic pollutants including dyes, metal ions, heavy metals, organic compounds, and other hazardous supplies. Therefore, the development of costeffective and efficient control methods against such pollution is vital to safeguard ecosystems and natural resources. In this regard, recent advances in science have propelled bioremediation as a potential alternative to conventional treatment methods. This review was structured to address bioremediation as a practical option for the treatment of textile dyes by evaluating its performance and typical attributes. It further highlights the present hurdles and future expectations for the removal of dye containing wastewaters via bioremediation techniques.

INTRODUCTION

Water is the most fundamental substance for all life on earth and a valued resource for human civilization. Reliable access to clean and affordable water is considered one of the most basic human requirements and remains a major challenge of this century. All over the world, nearly 780 million people still lack access to improved drinking water sources (WHO, 2011). Recovery and recycling of wastewater have become a growing trend in the past decade due to rising water demand, and water quality management has become a prime research interest due to water pollution problems. Besides the economy, the textile industry is one of the main reasons for creating wastewater effluent due to its high consumption of water for its different wet processing operations. This effluent discharge contains chemicals like acids, alkalis, dyes, hydrogen peroxide, starch, surfactant dispersing agents (Paul *et al.,* 2012). So, in terms of its environmental impact, the textile industry is estimated to use more water than any other industry, textiles mills of average size consume water about 200 L/kg in processing the fabrics

(Wang *et al.,* 2011; Kant, 2012). According to the World Bank estimation, textile dyeing, and finishing is given to a fabric that generates around 17 to 20 % of industrial wastewater, globally (Kant, 2012). Moreover, the rate of removal during primary and secondary treatments observed in textile wastewater treatment plants is low, due to their recalcitrant property (the breakdown of aromatic structures and amino groups), and it results in their easy carryover into the aqueous environment. As a result, the accumulation of these compounds can occur in soil sediments and extends to the drinking water supply chain (Salter-Blanc *et al.,* 2016; Xiang *et al.,* 2016). Synthetic dyes have been reported to be capable of generating aromatic compounds that show high toxicity with mutagenic and carcinogenic properties (Bafana *et al.,* 2011; Ito *et al.,* 2016). The uncontrolled discharge of textile waste water may cause severe health problems and shows higher impact on the environment due to their anaerobic degradation. They also cause a variety of other chronic effects (Bafana *et al.,* 2011).

Most textile wastewaters are highly coloured because they are typically discharged with dye contents in the range of 10-200 mg/L, and many

dyes are visible in the water at concentrations as low as 1 mg/L (Cervantes, 2009). These wastewater effluents often contain coloured substance along with hazardous chemicals which reduces the soil fertility when it gets discharged onto land (Pourbabace *et al.,* 2006) and also severely affects the photosynthetic function of plants. Synthetic dyes can affect plant growth by inhibiting seed germination, seedling survival rate, and elongation of shoot and root (Puvaneswari *et al.,* 2006). The presence of colour reduces the oxygen solubility (due to high chemical oxygen demand (COD)) and transparency of water due to low light penetration. They may also be lethal to aquatic organisms like daphnids, fishes, snails, and frogs due to the presence of component metals and chlorine (Bafana *et al.,* 2011). Dyes can also inhibit algal growth and photosynthesis by reducing the penetration of light (Weisburger, 2002). They also lessen the ability of algae to make food and oxygen. In addition, they also cause an ecological imbalance in other life forms (Rahimi *et al.,* 2016; Wang *et al.,* 2017). Not only plants and other organisms but humans are also greatly affected due to their toxicity.

Currently, there are stringent requirements for the discharge of textile effluents as it is unsafe for the environment and society. In the case of the colour of the textile, effluent is the primary concern due to its harmfulness to the environment and the public. In recent times, the recovery and reuse of wastewater have gained significant consideration because of the scarcity of water. Many different physio-chemical treatments are available to treat the wastewater and include adsorption, coagulation, membrane filtration, ion-exchange, sonication and plasma treatment. However, these methods have disadvantages, such as high operating/energy costs, production of large amounts of sludge (resulting in difficult handling and disposal), and production of damaging by-products (Robinson *et al.,* 2001). The physio-chemical methods are more costly, although the dyes are removed, accumulation of concentrated sludge may create a disposal problem. There is also a possibility that a secondary pollution problem will arise because of excessive chemical use. The interest today is not in technologies for colour removal but in technologies that can produce reusable water, remove toxicity, mineralizes aromatic compounds, and possibly no sludge production (Holkar *et al.,* 2016). In that view application of living organisms, such as plants and microorganisms for the treatment of wastewater, has gained importance

over other conventional processes. The utilization of microorganisms for the absorption and degradation of toxic chemicals present in the wastewater is a new way of treating toxic water. In this concern, the current paper aims to discuss the advancement in the application of microorganisms for the treatment of wastewater. Further, our intention extended to evaluate, whether the mixed microbial consortia have a greater ability than monocultures.

Biological systems involved in the discolouration of azo dyes

Due to a general concern about the treatment of wastewater, the laws are becoming more stringent, a large number of investigations have been recently developed to find more efficient methodologies for the treatment of wastewater. However, due to the complexity of the nature of pollutants, there is no single bioremediation technique that serves as a '*silver bullet'* to restore polluted environments. Microorganisms possess the versatile capability to remove the pollutants from wastewater by biodegrading the recalcitrant compounds (Mahmood *et al.,* 2016). Bioremediation using microbes such as fungi, yeasts, bacteria, and algae could not only decolourize several dyes but also completely degrade them under certain ecological conditions. Several recent investigations have been reported on microbiological approaches for dye decolourization (Song *et al.,* 2018; Mani *et al.,* 2019; Zahran *et al.,* 2019). Biological degradation methods can be classified based on oxygen requirements: aerobic, anaerobic, and anoxic (a combination of both aerobic and anaerobic methods) (Ahmad *et al.,* 2015). Generally, the anoxic method is widely used. In this method, the first anaerobic process has been used to treat dye wastewater with high COD, while the following one has been used for the resulting effluents with relatively low COD (Xiang *et al.,* 2016). Based on the mechanism of degradation of dye wastewaters, the degradation method can further divide into biosorption and biodegradation (Kaushik and Malik, 2009). In biosorption, microbial biomass can be used as a bio-sorbent. For example, fungi and algae are widely employed. Thiol, phosphate, amino, and carboxyl groups present in the cell walls of microbes bind to the azocompounds; this process of binding is fast and completed within a few hours of time (Chen *et al.,* 2016). In biodegradation, complete biodegradation occurs is said to be mineralization. In mineralization, organic compounds are converted

into water and carbon dioxide. The biodegradation process of textile dyes by bacterial strains can limit substrate diffusion into the cell, while fungal strains overcome this issue. Among various strains of fungi, the white-rot fungus has been found to be very effective at biodegradation (Ahmad *et al.,* 2015). Fungi produce ligninolytic enzymes that can bind a variety of textile dyes (Kaushik and Malik, 2009). Enzymes like lignin peroxidases (LiP), laccases, tyrosinases (Try), manganese peroxidases (MnP), NADH-DCIP reductase, azo reductase, hexane oxidases can be produced by diverse of organisms to reduce azo compounds (Lade *et al.,* 2015; Chatha *et al.,* 2017). Among these families of enzymes, laccases and azo reductases have shown a great potential to decolourize a broad range of known synthetic dyes. Occasionally, under unfavourable environment, some natural cellular enzymes may also get converted into dyedegrading enzymes, flavin reductase from *E. coli* acts as azo reductase (Russ *et al.,* 2000).

Dye decolourization by fungi

Currently, fungi have been proven to be useful in degrading and mineralizing recalcitrant textile dyes due to their powerful enzymatic machinery (extracellular ligninolytic enzyme system), morphology, and diverse metabolic capacity (Ahmad *et al.,* 2015; Rahimnejad *et al.,* 2015; Guo *et al.,* 2020). The mechanism of fungal degradation involves adsorption and enzymatic degradation or a combination of both. Bio adsorption plays an important role in the decolourization of dyes by living fungi and also enzymatic degradation, which includes different enzymes such as azo reductases, laccases, Manganese peroxidases, and lignin peroxidases. Thus the exact mechanism of azo dye decolourization is still unknown. Various reports have been submitted by researchers on fungal biodegradation is mentioned in Table 3. Fungal biodegradation, which includes so many factors such as good optimal conditions, temperature, time of decolourization, etc., with the optimal conditions, more than 90% of colour reduction, can be achieved. Fungi can be used in the decolourization process, but they have low pH stability, and thus this is considered a major disadvantage. For instance, Wang *et al.* (2017) reported decolourization and degradation of Congo red using *Ceriporialacerata* a white-rot fungus isolated from decayed mulberry branches. This study showed 90% degradation of

Congo red dye with 48 h.

Dye decolourization using yeast

Yeast decolourization and degradation of dyes have not been extensively studied. Yeast has mainly been studied with Biosorption mechanisms. Yeasts have been employed in the decolouration of different azo dyes because they have many advantages in bioremediation purposes, such as accumulation of dyes in high capacity and heavy metals, such as lead and cadmium (II) (Fairhead and Thöny-Meyer, 2012), fast growth and decolouration than filamentous fungi and the ability to survive under unfavourable conditions (Martorell *et al.,* 2012). Sludge from wastewater harbours wide varieties of yeast strains compared to other environments, although yeasts are still a negligible fraction of the microorganisms present in activated sludge. The mechanisms of yeast can involve adsorption, enzymatic degradation, or a combination of both in decolourization processes (Grassi *et al.,* 2011; Tan *et al.,* 2013) yeast can withstand very low pH. Comparing decolourization activity in terms of low pH, yeast is much capable than fungi during the bioremediation of textile dyes. Yeast can also undergo enzymatic degradation with more stability. Information on the use of yeast to decolourize dyes is presented in (Table 4).

Dye decolourization by algae

Algae are ubiquitous and are getting an increasing concern in the area of degradation of textile dyes. Several species of algae that have been successfully used are reported in Table 5. Algae act as adsorbent comparing with commercial synthetic adsorbents and treat higher volumes of dye wastewater because of higher biomass content, and also, they can undergo biodegradation mechanisms. Processing and utilization of algae is easy than other organisms. A literature review recommends that degradation of dyes by algae occurs through three different mechanisms such as a) utilization of dyes for their growth, b) transformation of dyes into other intermediates or $CO₂$ and water c) adsorption of chromophores on algae. Biosorption and biodegradation are very dissimilar phenomena. Biosorption implies adsorption of dyes from water to solid phase (bio-adsorbents), while biodegradation means the transformation of one compound to another by enzymes.

Table 3. *Continued ...*

Keys: Lignin peroxidases (LiP), Laccases, Tyrosinases (Try), Manganese peroxidases (MnP)

Dye decolourization by bacteria

Usually, azo dye decolourization occurs under aerobic, anaerobic and facultative anaerobic conditions by diverse groups of bacteria. Anaerobic bacteria highly achieve colour removal by producing azo reductases that cleave azo bonds (–N=N–) and result in the formation of aromatic amines, which is mutagenic in nature (de Souza *et al.,* 2019; Fu *et al.,* 2019). Under anaerobic conditions, the azo dyes act as a terminal electron acceptor (de Souza *et al.,* 2019). In contrast, activated sludge processes being aerobic, but they do not actively involved in the colour removal process (Roy *et al.,* 2018). Many research studies have been carried out to find out the role of various groups of bacteria in the decolourization of azo dyes in monocultures (table 6) and by a mixed consortium (Table 7). The bacterial decolourization has gained interest since it can reach a higher degree of biodegradation and mineralization, applies to a wide variety of azo dyes which is inexpensive and biodegradable, and produces less sludge and said to be environmentally friendly (Saratale *et al.,* 2009) and also bacterial degradation is considered much faster than all other organisms. They have high stability in terms of pH and can thrive under stressful conditions also. Bacterial degradation can be of either usage of pure cultures or mixed cultures.

Application of microbial consortia-based bioremediation

Monocultures can decolourize only a narrow range of different azo dyes and has less efficiency to degrade it completely (Khan *et al.,* 2014). The use of mixed microbial inhabitants for dye wastewater treatment is essential to achieve an elevated rate of decolourization, degradation, and mineralization. Synergistic activities of mixed cultures during the degradation of synthetic dyes have advantages over the usage of monocultures (Raman and Kanmani, 2016; Shah *et al.,* 2016). In the microbial consortium, the individual strains may attack the dye molecule at a different position or make use of other metabolites produced by the co-existing strains for further decomposition. In such cases, microbes adapt themselves to the toxic wastes and develop resistance naturally, which then transform various toxic chemicals into less toxic ones (Ratho *et al.,* 2013). Several enzymes produced by the consortium may lead to complete degradation of chemical compounds (Holkar *et al.,* 2016). Cleavage of azo bond results in aromatic amines generation is often toxic. However, in a mixed culture, these aromatic amines can be degraded by the synergistic activity of microbes. The bacterial consortium is the most often used for decolourization of azo dyes, as they are usually fast to multiply rapidly under aerobic, anaerobic, anoxic conditions, as well as in extreme

Table 4. Dye decolourization using yeast

stress conditions, like high salinity and extensive variations in both pH and temperature (Holkar *et al.,* 2016). The efficiency of decolourization of the consortium is compared to monoculture removal is may be due to the involvement of quorum sensing: the mechanism by which bacteria regulate their gene expression within their population. Quorum sensing allows bacterial populations to communicate and organize group activities. Recent studies regarding the biodegradation of dyes using bacterial consortia are reported in Table 7. The bacterial decolourization can be directly influenced by various factors (Netzker *et al.,* 2018) such as the temperature, pH, dye structure, inoculum concentration, dye concentration, different carbon

and nitrogen sources, electron donor, agitation speed, oxygen transfer rate, redox mediator (Alvarez *et al.,* 2016) and NaCl concentrations. Therefore, adapted bacterial strains, isolated from dye contaminated sites, are very efficient in the removal process due to adaption to different extreme environmental conditions (Holkar *et al.,* 2016). In contrast to bacterial consortium degradation, few reports are available on decolourization of dyes by yeast, algal, fungal consortia also(Ito *et al.,* 2016; Ghosh *et al.,* 2017). Another approach is the synergistic action of a fungal-bacterial consortium, which provides an alternate approach for the efficient removal of various contaminants. Some reports indicated that

Table 6. Dye decolourization by monocultures

fungi-bacteria co-cultures showed higher efficiency and stability (Yuan *et al.,* 2018) to degrade the mixture of many kinds of macromolecule organics into small-molecule substances, which can be further degraded or even mineralized by bacteria (Lade *et al.,* 2016).

Challenges faced and future prospects

Bioremediation is a widely used effective method for the treatment of dye effluents. Though they have significant positive aspects, there are many challenges that have to be discussed. The main problem of textile effluents is colour, COD, and other non-biodegradable matter. It is now possible to reduce BOD and COD in textile wastewater by practically available methods. Nevertheless, in the case of other substances, the bioremediation lacks the capability to treat them. In those cases, bioremediation can be used in combination rather than a single method of treatment. Thus, this would help in removing the pollutants from the wastewater. The presence of aromatic amines after treatment will also affect the quality of treated wastewater because they are very diverse in their chemical nature so that biological treatment would not remove all amines present in the textile dye waters. The excess sludge production is also a major problem, which should take into account. This would cause a problem for the environment in many aspects. So, designing and implementing combined treatment is necessary, which also should focus on treating real textile effluents in the future.

CONCLUSION

Bioremediation is an eco-friendly approach to alleviate the impacts and toxicities of the intermediate metabolites produced by textile effluents. Bioremediation using pure cultures and consortia provides an efficient and trustworthy way to mineralize and reduce the toxicity of dyes. They use various mechanisms to detoxify dyes, especially enzyme-mediated bioremediation. At present, the design of such consortia is gaining importance as

Mixed bacterial cultures	Source	Dyes and concentration	Mechanism and optimal conditions	Decolourization rate $(\%)$	Reference
Pseudomonas sp. ARa Bacillus sp. ARc Bacillus sp. ARd) and Ochrobactrum sp. ARf (Consortium-AR1)	Soil sample	Reactive Red 195 (100 ppm- 2000 ppm)	Degradation; pH 8, 40 °C, 14-92 h under static conditions.	99-45% colour	Khan et al., 2014
Citrobacterfreundii - SUB 3, Moraxella osloensis - SUB 4, Pseudomonas aeruginosa - SUB 10, Citrobacter freundii - SUB 6, Pseudomonas aeruginosa BL22 (Consortia CN-1)	Domestic Sewege	Mordant black 17 (100 ppm)	Degradation; pH 7.5, 37 °C, 14-48 h under shaking conditions	95% colour (100ppm)	Karunya et al., 2014
Providencia rettgeri strain HSL1 and Pseudomonas sp. SUK1	Dye contaminated soil	Reactive black 5, Reactive orange 16, Disperse red 78, Direct red 81 (100ppm)	Degradation; pH 7, 30 °C, $12-30$ h; azo reductase, NADH-DCIP reductase	RB 5 (98 % in 30 h), RO 16 $(99\% \text{ in } 12 \text{ h})$, DR 78 (98 % in 18h) and DR 81 (99%) in $24 h$)	Lade <i>et al.</i> , 2015
Bacillus subtilis, Bacillus cereus, Bacillus mycoides Bacillus sp., Micrococcus sp., and Pseudomonas sp. (Consortia-BMP1/ $SDSC-01$	Wastewater, sludge, and soil	Carmine Red, Light Green, Eriochrome Black T, Metanil Yellow (200ppm)	Degradation; pH 7.5, 37 °C, 24 h	Carmine Red- 93%, Light Green-94%, Eriochrome Black T-93%, Metanil Yellow- 94%, mixture of dyes-89% colour	Mahmood et al., 2015
Pseudomonas aeruginosa, Bacillus flexus and Staphylococcus lentus	From a collection	Acid blue 113 (600ppm)	Degradation; 36 °C, 16 h	93.7 % colour	Shanmugam and Mahadevan, 2015
Citrobacterfreundii - SUB 3, Moraxella osloensis - SUB 4, Pseudomonas aeruginosa - SUB 10, Citrobacter freundii – SUB 6, Pseudomonas aeruginosa BL22 (Consortia CN-1)	Domestic Sewege	Acid blue 113, Acid black 24, Mordant black 17 (100ppm)	Degradation; pH 6-9, 20-40 \degree C, 24-48 h	93.34% colour, after 48 h	Nachiyar et al., 2016
Bacillus flexus strain NBN2, Bacillus cereus strain AGP-03, Bacillus cytotoxicus NVH 391-98 and Bacillus sp. L10	Soil samples	Direct Blue 151 (DB 151) and Direct Red 31 (DR 31) (200ppm).	Degradation; pH 9.5, $30-36$ °C, 120h	DB 151 and DR 31 up to 97.57% and 95.25 at 36°C	Lalnunhlimi and Krishnaswamy, 2016

Table 7. Dye decolourization by mixed bacterial cultures

Table 7. *Continued ...*

they work under adverse and stressful conditions. Further, optimizations of various parameters are required for better utilization of consortia and maintain environmental safety and health of all life forms.

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