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REVIEW ARTICLE

Plant-microbe-dye interaction during rhizoremediation

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Abstract

The conventional technologies used for textile dye wastewater treatment are costly, and involve high energy consumption and sludge production. However, bioremediation using plants and microbes involves low cost, energy consumption, and no sludge formation. Hence, the review focuses on the dye degradation by the plant-microbe treatment system.

Keywords: conventional technologies, bioremediation, low-cost

Introduction

The low-cost perspective of the treatment system for mixed pollutants was the utilization of plant-microbe combinations (Jayapal *et al.*, 2022; Supreeth *et al.*, 2021). Such a system demonstrated efficiency to remove Chemical Oxygen Demand (COD), ammonical nitrogen, and phosphorous in the eutrophicated water (Supreeth *et al.*, 2021). Although physicochemical methods efficiently remove high-level pollution, the process is energy-consuming and intrusive to the environment. The advantages of the plant-microbe integrated system in pollutant removal are owing to its cost-effectiveness and almost nil sludge formation. The rhizoremediation technique is used, when the treatment is less urgent and when the contamination level is moderate (Segura & Ramos, 2013). Understanding the interaction between plant and microbe during rhizoremediation is significant in optimizing process parameters; hence, the review focuses on the overall mechanism involved during the treatment process.

Plant-Dye interactions during rhizoremediation

Root exudates (organic molecules) released in the rhizosphere increased pollutants' bioavailability to the microbes, thereby facilitating their degradation (Luo *et al.*, 2017). The succinic acid and glucose present in the exudates of the Barmultra plant degraded the pollutant naphthalene when combined with the microbe *Pseudomonas putida* (Kuiper *et al.*, 2002). Root exudates that remediate dyes were also reported. For instance, *Portulaca grandiflora* Hook exudates removed Navy Blue HE2R dye (Khandare *et al.*, 2011). The exudate composition depends on the plant variety and the nature of the pollutants (Phillips *et al.*, 2012). The hydrocarbons act as substrate for microbes and decrease. Besides, the phenolic compounds increased with PAH stress (Liu *et al.*, 2022). The degradation of phenol, petroleum compounds and benzo[a]pyrene by the microbes utilizing sugars, palmitic acid and phenolic compounds in the root exudates (Jin *et al.*, 2019; Liu *et al.*, 2015; Toyama *et al.*, 2011).

Dyes are one major category of pollutants. The mechanism of plant-dye interaction occurs by adsorption/absorption, translocation, and transformation of dye molecules inside plant cells.

Adsorption or absorption of dye molecules onto the plant surface

Plants can adsorb dye molecules onto their roots. The adsorption of dye by *Leucaena leucocephala* roots was earlier reported (Bharathiraja *et al.*, 2018; Jayanthy *et al.*, 2014; Jayapal *et al.*, 2018). The removal of pollutant dye could be either through adsorption or absorption. The hydrophobicity of the molecule determines the process. The octanol-water partition coefficient (log K_{ow}) determines the uptake of organic molecules. When log $K_{ow} < 1$ for dyes molecules, it enters plant roots and translocates. If, log $K_{ow} > 3.5$, as for complex dye molecule, it adsorbs onto the roots (Christian & Beniah, 2019). The dye molecules are absorbed and metabolized by plants. They also accumulate dyes within the intracellular spaces, resulting

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in dye decolorization by the enzymes, mainly laccases and peroxidases (Uruj *et al.*, 2015). *Eichhornia crassipes* removed most of the Black B and Red RB dye by its adsorption onto plant parts. Dye absorption and accumulation mechanism removed dyes by the fibers in banana (Muthunarayanan *et al.*, 2011; Abdul Karim *et al.*, 2016; Siti *et al.*, 2018). Many other plants efficiently removed textile dyes in the industrial effluent by absorption (Ahila *et al.*, 2021).

Translocation and transformation of dye molecules

Transformation of pollutants occurs in three phases as follows (Kvesitadze *et al.*, 2009). In the functionalization process, the hydrophobic molecules acquire hydrophilicity by enzymatic transformations such as oxidation, reduction, and hydrolysis. In conjugation, xenobiotics were chemically coupled with proteins, organic acids, and polysaccharides to form peptide, ether, and ester with covalent bonds and increase the hydrophilicity of the molecules. At the final stage of compartmentation, the conjugates accumulate in vacuoles and insoluble conjugates get excreted out of plant cells' vital organs (Kvesitadze *et al.*, 2006). The plant cell's nuclei, swollen mitochondria, enlarged plastids, and widening of ER and Golgi apparatus were observed when the phytoremediator accumulated dye molecules (Prasad & Aranda, 2018). The withstanding capacity of the plant cells determines their detoxification potential. The degradation of dye molecules in plant leaves was reported (Shahi *et al.*, 2018) (Figure 1).

Plant-microbe interactions in the rhizosphere and abiotic stress tolerance

Abiotic stress, which leads to agricultural productivity loss, can be mitigated by the addition of PGPR. The abiotic stress influences the plant-microbe association by the altered root exudates or microbial activity in the rhizosphere (Tripathi *et al.*, 2015). Upon abiotic stress induction, the microbes interact with both the plant and other microbes and induce functional catabolic genes in the microbial community, and lead to alleviation of pollutants (Segura & Ramos, 2013). The microbial species *Trichosporon akiyoshidainum* from the rhizosphere of the plant *Cinnamomum porphyria* degraded azo and anthraquinone dyes (Nanjani *et al.*, 2021). In general, PGPR ameliorated abiotic stress responses through phytohormones (IAA, ABA) production, antioxidant production, and degradation of ethylene precursors (Michael *et al.*, 2021). Adopting this mechanism, PGPR alleviated stress due to hydrocarbons (organic compounds), and metals such as chromium in the plant andimproved its growth (Tirry *et al.*, 2021; Vocciante *et al.*, 2022). When microbes are exposed to nutrient deficit and pollutants-rich environment, they adapt to the pollutants, thrive in toxic conditions, and form a functional community by horizontal gene transfer mechanism to remediate the pollution (Gkorezis *et al.*, 2016). In case of dyes the rhizosphere possesses microaerophilic conditions and hence the aerobes can break down dye molecules using oxygenases and utilize them in their metabolism (Figure 2).

Microbe-Microbe Interaction During Rhizoremediation

The microbe-microbe interaction is influenced by quorum sensing (QS) and by competition for nutrients (Harris *et al.*, 2020). QS of microbial biofilm stimulates bioremediation by binding to transcriptional factors and initiates the cascading of appropriate catabolic genes. The microbe interacts with other microbial species in the rhizosphere, causing aggregationand horizontal gene transfer to form a functional microbial community (Yin *et al.*, 2021; Segura & Ramos, 2013). The biofilm's exopolysaccharide (EPS) increased the solubility and enhanced pollutant degradation (Kumari *et al.*, 2016). The biofilm increases the cell density around the xenobiotics, thereby removing pollutants (Maddela *et al.*, 2019). The microbes *Vibrio fischeri* removed Reactive brilliant red X-3B, *Bacillus amyloliquefaciens* degraded crystal violet, *Bacillus sp. AK1* and *Pseudomonas aeruginosa* degraded Amaranth and malachite green dyes

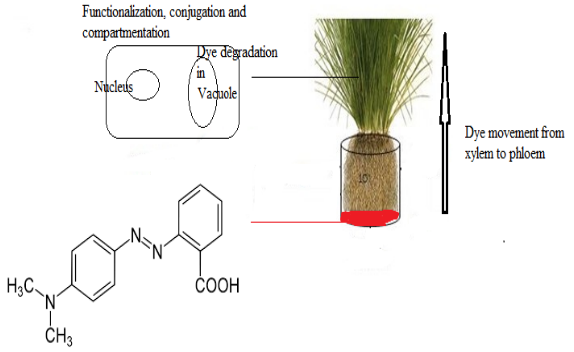

Figure 1: Schematic representation of dye-plant interaction during the rhizoremediation process

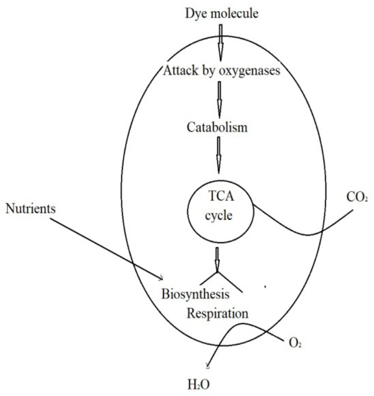

Figure 2: Aerobic catabolism of dyes by microorganisms (Gkorezis *et al.*, 2016).

respectively (Liu *et al*., 2017; Sun *et al*., 2016; Watharkar *et al*., 2013; Kalyani *et al*., 2012). The remediation of dyes was controlled by factors, namely pH, dissolved oxygen, type and concentration of dyes, redox potential, and chemotaxis ([] Mohapatra *et al*., 2019).

Dye-Microbe interaction in the rhizosphere

The microbe adsorb onto the dyes using covalent, electrostatic interaction was earlier reported (Li *et al*., 2019). The adsorption capacity of the dye onto microbial surface was earlier researched by pseudo kinetics (Sari *et al*., 2019). The adsorption by physisorption follows the pseudo-first order and chemisorptions follows pseudo second order model (Simelane *et al*., 2018).

Dynamic interaction of plant-microbe-dyes during rhizoremediation

The interaction between plant and microbes under the abiotic stress condition is dynamic. Root exudates (mainly flavonoids) act as carbon or nitrogen source for microbes, alter pH and make pollutants available to the microbes. Microbes, in turn, modify the constituents of root exudates by releasing volatile organic compounds (VOCs), microbe-associated molecular patterns (MAMPs), and Nod and Myc factors (Ma *et al*., 2016). Plants enrich specific microbes based on the constituents in the exudates, promote chemotaxis, distribution along the roots, and form a symbiotic relationship (Haichar *et al*., 2008). For the adsorption and root colonization, the protein and lectin were involved. Besides, plants recognize N-acetyl-L-homoserine (AHL) from bacterial QS and modulate gene expression important for manifesting defense or degrading mechanisms (Mangwani *et al*., 2016; Shrestha *et al*., 2020).

Under stress conditions, rhizospheric microbes produce IAA, cytokinins, and gibberellic acid. IAA from both plants and microbes induces root exudation and growth of lateral roots. ACC deaminase-producing PGPR nullifies the abiotic stress-induced ethylene production in plants and inhibits damage to the plant (Ali *et al*., 2014).

The roots facilitate the colonization by beneficial bacteria by their exudation pattern and avoid pathogenic bacteria. Plants, while preventing pathogens, also stimulate beneficial microbes and balance them with MAMPs. Molecules in the jasmonate signaling pathway in pollutant degradation were reported earlier (Ma *et al*., 2021).

The coordination between pathogens and beneficial microbes occurs at the spatial and temporal level and increases the resilience of plant species. The plant-microbe follows four stages of interaction using the Zigzag model. At stage I, pattern recognition receptor (PRR) recognize the microbe's Pathogen-associated molecular pattern (PAMP) and prevent its entering. In stage 2, the transportation of effector molecules and induction of effector-triggered susceptibility (ETS) in plants. At stage 3, the intracellular receptors (R proteins) identify particular effectors and induce effector-triggered immunity (ETI), which induces programmed cell death. At stage 4, the pathogen escapes ETI and leads to ETS (Dogra *et al*., 2018).

The abiotic stress-induced pathways, mainly jasmonic acid (Yuan *et al*., 2008), which paved way for transcription of corresponding genes and induces a defense mechanism. Rhizosperic microbes increased the bioavailability of pollutants to plants as well as degraded them (Gayathiri *et al*., 2022).

The inoculation of appropriate microbes induces plant biomass along with rhizoremediation (Ojuederie *et al*., 2017). The dye remediates microbes *Klebsiella sp. VITAJ23* was used as bio inoculant and facilitates remediation of reactive green dye when used with *Alternanthera philoxeroides* plantlets (Astha *et al*., 2019). The bacterial species *Bacillus, Lysibacillus*, and *Pseudomonas* were involved in root colonization of *Phragmites australis* during the dye pollutant degradation (Riva *et al*., 2019). The plants remediate toxic dyes by their adaptation to the polluted environment before treatment (Bharathiraja *et al*., 2018).

Discussion

The process of using plants and their symbiotic associates i.e. rhizospheric bacteria for the removal of pollutants from waste products, especially the industrial effluents containing textile dyes, chemicals, metals and their salts is known as phytoremediation. It is important as these pollutants are toxic, carcinogenic and mutagenic (Khandare *et al*., 2012). The physicochemical processes of treating these wastewater are rather expensive and produces a large amount of sludge. A number of studies are now being done to produce plant-microbe based system to remove these pollutants from the water and decrease its toxicity.

Abdul Karim *et al*. (2017) tried to remove crystal violet dye and acid green (AG) dyes from aqueous solution with the help of banana. Both the dyes were successfully removed with increasing intensity upto a saturation point. Effect pH was also studied and found to be effective for only AG dye. Watharkar *et al*. (2013) made use of bacterial biofilms for the extraction of dyes from industrial waste materials and found that Amaranth dye can be decolorized easily even at 600 mg $l^{-1,}$ which was confirmed by the spectrometer.

Watharkar *et al*. (2013) decolorized Navy Blue RX (NBRX) by using *Bacillus pumilus* strain PgJ isolated from *Petunia grandiflora* Juss. Mix of both could decolorize the NBRX up to 96.86% within 36 h.

Astha (2019) removed 79% of reactive green dye with increased enzyme activity. The pot culture indicated that *A. philoxeroides* was very tolerant to this dye, even upto 3000 mg L^{-1} of dye concentration.

Tara *et al*., (2021) found that bacterial-augmented floating treatment wetlands (FTWs) with three dye-degrading bacteria *Acinetobacter junii* strain NT-15, *Pseudomonas*

Table 1: I) Pollutant (dye) removal using plant-microbe integrated system

S.No	*Plants-bacteria consortium*	*Dyes*	*References*
1.	*Phragmitesaustralis – Pseudomonas, Acinetobacterjunii, Rhodococcus sp.*	95.5% of Reactive Black 5	Tara *et al.*, 2021
2.	*Vetiveriazizanioides-microbial consortium*	78.8% of Mixed dye containing textile effluent	Jayapal *et al.*, 2022
3.	*Zinnia angustifolia* in consortium with *Exiguobacterium aestuarii* strain ZaK	100% of Remazol Black B	Khandare *et al.*, 2012
4.	*Pseudomonas sp-Phragmitesaustralis*	Reactive Black (RB) 5, Blue S2G dyes	Riva *et al.*, 2019
5.	*P. grandiflora* and *B. pumilus* consortium	96.86% of Navy-Blue RX	Anupritaet *al.*, 2013
6.	*Ipomoea hederifolia* and its endophytic fungus (EF) *Cladosporium cladosporioides*	97% of Navy Blue HE2R	Swapnilet *al.*, 2016
7.	*Tagetus patula, Aster amellus, Portulaca grandiflora, Grindelia grandiflora*	73% textile dye effluent	Chandanshiveet *al.*, 2018
8.	*Alternanthera philoxeroides - Klebsiella sp. VITAJ23*	79% of reactive green dye	Astha *et al.*, 2019

indoloxydans strain NT-38, and *Rhodococcus* sp. strain NT-39) and the plant *Phragmites australis* could degrade an azo dye, Reactive Black 5 (RB5) successfully (95%). Also, the metabolite thus formed were found to be not harmful to the faunal population.

Jayapal *et al.* (2020) studied the degradation of industrial textile-dye waste degradation by using a sequential anaerobic-aerobic plant-microbe system with vetiver plants. They found that the system succeeded in decolorizing dye (78.8%) and removing of total aromatic amine (TAA) (69.2%). Also, it reduced the toxicity and teratogens. Khandare *et al.*, 2012 studied phytoremediation by utilizing consortiuom of plant *Zinnia angustifolia* and the bacterium *Exiguobacterium aestuarii* strain ZaK on the removal of Remazol Black B dye (RBB) and found it to be more efficient in the degradation of dye as compared to the bacterium and the plant when used separately.

Some of the other studies experimenting on dye pollutant removal by various integrated plant-microbe systems are given in Table 1. Since extremely large amounts of dyes like toxic azo dyes and other contaminants are discarded in the sewage system, which are precarious to the health of living organisms and many of which do not degrade by themselves because of their complex structural configuration, it is the need of time to develop methods to sustainability remove them from the water to save faunal and human population from their harmful effects.

Conclusion

The dynamic interaction in the rhizosphere induces the development of a specialized microbiome and aid in the subsequent pollutant removal. Hence, understanding the mechanism helps to remove various pollutants by the rhizoremediation treatment system.

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