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# Bacterial Augmented Floating Treatment Wetlands for the Remediation of Dye Contaminated Effluent

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## ABSTRACT

Advancements in the industrial sector have increased water contamination due to the random discharge of untreated industrial effluents, causing hazards to all living beings. Recently, floating treatment wetlands (FTWs) have been shown to be an inexpensive, novel, and eco-friendly technique to treat wastewater. The current research is focused on examining the azo-dye decolorizing ability of *P. australis* (Common Reed) augmented with a bacterial consortium (*Bacillus* sp. and *Acinetobacter* sp.) in FTWs. Bacterial augmentation significantly enhanced dye decolorization and improved the removal efficiencies of key physicochemical parameters, including chemical oxygen demand (up to 94%), biological oxygen demand (up to 91%), total dissolved solids (up to 96%), and total organic carbon (up to 92%), compared to non-inoculated systems. In addition, bacterial inoculation alleviated phytotoxic stress and promoted plant growth and biomass production. The results demonstrate that plant-microbe synergism substantially improves FTW performance and highlights the potential of bacterial-augmented FTWs as a sustainable and scalable approach for textile wastewater remediation.

## 1. INTRODUCTION

Water pollution has increased mainly due to industrialization, with industrial effluents randomly discharged into water bodies without prior treatment (Singh, 2024). Textile industries, particularly during dyeing, release heavy metals like copper, chromium, nickel, and lead, which are toxic and carcinogenic (Chaurasia *et al.*, 2023). This pollution causes an imbalance in the environment, increasing water's chemical oxygen demand (COD) and biological oxygen demand (BOD), altering its pH, changing its organic-inorganic chemical content, and damaging many fauna and flora, thereby posing a serious threat to the aquatic ecosystem sustainability (Dhameliya and Ambasana, 2023).

Despite its significant economic role, the textile industry is also a major environmental concern. The World Health Organization (WHO) states that 17-20% of industrial water pollution originates from dyeing treatment in the textile industry. Approximately 80% of the dyes used in the industry are azo dyes, and about 10-15% of these dyes are released into the environment as effluent without binding to fibers (De Campos Ventura-Camargo and Marin-Morales, 2013). Azo dyes have one or more azo bonds (-N=N-) which join the different aromatic structures. Breaking these bonds, either biologically or chemically, can release harmful byproducts that are highly mutagenic and toxic (Alderete *et al.*, 2021).

Azo dyes, known for their ease of use and cost-effectiveness, are extensively used in various industries including textiles, rubber, enamel, plastics, cosmetics, etc. (Kumar *et al.*, 2021). However, these dyes pose significant health risks as they are toxic, mutagenic, and carcinogenic, impacting not only aquatic life but also humans who are exposed to these dyes or colored effluents (Kim *et al.*, 2024). Due to their large-scale production and widespread application, azo dyes are readily released into environmental waters and hence, they are considered micropollutants in aquatic ecosystems (Hashemi and Kaykhahi, 2022). Developing countries also use water from contaminated water bodies for crop irrigation (Verma *et al.*, 2022). Ultimately, when humans use polluted groundwater and food, they cause serious health hazards by leading to diseases like cancer, skin allergies, etc. . Removal of azo dye from wastewater is essential due to its toxicity and potential to transform into harmful compounds in the human body (Pay *et al.*, 2023).

Conventional wastewater treatment methods usually pose a significant challenge to degrading complex pollutants, like aromatic compounds used in textile dyes. Methods such as membrane bioreactors, floatation, chemical oxidation, adsorption, and coagulation can be proposed; they can produce secondary pollutants and large quantities of sludge (Choubey and Shukla, 2019). Azo dyes can break down into more toxic aromatic amines by the action of microbes (Alzain *et al.*, 2023). Even treated industrial wastewater can contain heavy metals and harmful substances causing risks to soil and crops (Ofori *et al.*, 2021). This shows the necessity of treating wastewater to eliminate environmental and health problems. An innovative and cost-effective approach to treating wastewater is FTWs. Planted with aquatic plants, these wetlands can effectively remove contaminants from water, becoming a positive alternative to

traditional methods (Sahreem and Mukhtar, 2024).

Various researchers have applied FTWs in their studies to treat wastewater, such as domestic wastewater, stormwater management, and petroleum hydrocarbon-contaminated water (Shen *et al.*, 2021). The plants showed poor wastewater treatment ability because toxic compounds in wastewater affect plant growth. A synergistic combination of plant and pollutant-degrading bacteria like rhizobacteria and/or endophytic bacteria has been suggested (Sahreem and Mukhtar, 2023). The research revealed that FTWs planted with *Eichhornia crassipes* and *Pistia stratiotes*, and inoculated with *Bacillus* bacteria, effectively minimized pollutants in textile wastewater (Zahari *et al.*, 2021).

Many plant species such as *Phragmites australis*, *Typha angustifolia*, and various *Scirpus* species are generally utilized in floating treatment wetlands. The phytoremediation properties of these plants remove contaminants and assimilated dyes to manage textile wastewater (Hoang *et al.*, 2024). Many studies such as Abed *et al.* (2019), Rehman *et al.* (2018), and Mohsin *et al.* (2023) have shown that *Phragmites australis* have phytoremediation potential for wastewater treatment. The research highlighted that a 50% coverage of *Phragmites australis* in floating treatment wetlands had excellent results for treating wastewater. Maximum removal of BOD up to 53% and COD up to 50% were achieved (Yadav *et al.*, 2023). The study in the Arabian Peninsula demonstrated that *Phragmites australis* is suitable for removing heavy metal polluted wadis. The plant successfully collected heavy metals like Cd, Zn, and Pb (Al-Homaidan *et al.*, 2020). Another study showed that *Phragmites australis* serves as a medium for diverse bacteria in its root zone. Bacteria, because of symbiotic relationships played a key role in nutrient cycling and pollutant breakdown in



constructed wetlands. The microbial communities in the plants are enriched due to wastewater exposure, increasing their ability to treat wastewater (Cangioli *et al.*, 2022). All these studies revealed the phytoremediation ability of the aquatic plant *Phragmites australis* for wastewater treatment and its safe use for later purposes.

*Phragmites australis*, a local aquatic plant of Lahore, Pakistan, was selected for this research. The capability of *phragmites australis* for the eco-friendly elimination of Azo dyes using FTWs has not yet been explored. This study is probably the first to investigate the green removal of azo dyes from water with the help of a bacteria-augmented floating treatment wetland system. The current research focuses on increasing the efficacy of pollutant removal by FTWs by incorporating a specialized dye-decolorizing and plant growth-promoting bacterial consortium, including strains SZ-1 and LGW6. The degradation of azo dye compounds is facilitated by symbiotic interactions between the plant root and microbial community and ultimately improve water quality. Parameters that may affect the performance of FTWs were also examined. This innovative solution not only deals with the critical concern of textile dye pollution but also supports a sustainable and cost-effective wastewater processing system, emphasizing the prospect of FTWs in pollution control.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Wastewater was collected from an industrial site located in Lahore. A stock solution of azo dyes was prepared by dissolving 1 g of azo dye powder in 100 mL of distilled water. These dyes were selected because they are known for their ease of use and cost-effectiveness. However, these dyes pose significant health risks as they are toxic, mutagenic, and carcinogenic.

### 2.2 Bacterial Strains and Plant Selection for Dye Degradation

Two bacterial strains, SZ-1 (*Bacillus* sp.) and LGW6 (*Acinetobacter* sp.) were used to degrade azo dyes. *Phragmites australis*, commonly known as the common reed, was chosen to develop FTWs due to its ability to improve water quality by absorbing contaminants. This plant was subjected to various concentrations of dyes and pollutants in water under natural conditions. After a 20-day experimental period, observations were made on plant biomass and the lengths of roots and shoots.

### 2.3 Evaluation of Bacterial Growth on Dye Augmented MSM and Strains Compatibility

Bacterial strains SZ-1 and LGW6 were aseptically streaked on minimal salt medium (MSM) agar plates augmented with 25 ppm of the azo dyes. The plates were incubated at 37°C for 48 hours. To assess the compatibility of the bacterial strains, SZ-1 and LGW6, they were cross-streaked on the same nutrient agar plate and incubated at 37°C for 48 hours. Both strains were grown in flasks and mixed in equal proportions to make the bacterial consortium

### 2.4 Dye Decolorization Assay

After the compatibility test, bacterial strains were cultured separately in 30 ml of MSM broth and 25 ppm of azo dye at 37°C for 6 to 7 days and 120 rpm. The azo dye decolorization and degradation capabilities of the two bacterial strains, SZ-1 and LGW6, were measured spectrophotometrically.

### 2.5 Development of Floating Treatment Wetlands (FTWs)

The FTWs were developed to enhance the treatment of contaminated water. Polystyrene sheets were cut into circular shapes approximately 18 inches in diameter. Each mat featured a hole, and thirty-six mats were



created for four different microcosm treatments. These mats were then planted with *P. australis* seedlings, with nine mats receiving uniform plant seedlings. The Floating Treatment Wetlands (FTWs) were placed in plastic containers; each filled with 3 L of tap water. The plants were allowed a month in the tap water to develop their roots. Once optimal root growth was achieved, the tap water was replaced with water contaminated with dye. Each treatment, conducted in triplicate, was run under natural environmental conditions at UMT. The experiment employed the following treatments:

*Control 1:* Uncontaminated water with vegetation (*P. australis*) and no bacterial inoculation

*Control 2:* Dye-contaminated water

*Treatment 1:* Dye-contaminated water with vegetation

*Treatment 2:* Dye-contaminated water with vegetation and bacterial inoculation

*Treatment 3:* Dye-contaminated water with bacterial inoculation

## 2.6 Sample Analysis

Water samples were initially collected immediately (0 hr) after inoculating the bacterial inoculum into the dye-contaminated water for analysis.

## 2.7 Physical Properties of Contaminated Water

The physical properties of the contaminated water, such as pH, electrical conductivity, color, and temperature, were analyzed. The electrical conductivity of the samples was analyzed using a benchtop digital EC meter.

## 2.8 Chemical Parameters

The chemical properties were measured through the following analyses of the water samples:

**2.8.1 Chemical Oxygen Demand (COD):** To determine the COD, a sample was taken, and a known quantity of potassium dichromate and a catalyst, usually silver sulfate ( $\text{Ag}_2\text{SO}_4$ ), was added. The COD concentration was determined by heating the sample at 150-160°C for a specific period (typically 2 hours) in a reflux apparatus to oxidize organic compounds. The excess dichromate was titrated with a reducing agent, usually ferrous ammonium sulfate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ), in an acidic medium.

**2.8.2 Biological Oxygen Demand (BOD):** Biological Oxygen Demand (BOD) was determined by determining the initial dissolved oxygen (DO) levels in water samples followed by a five-day incubation at 20°C in the dark to facilitate microbial decomposition. The BOD value was measured by subtracting the final DO from the initial DO. This difference represents the amount of oxygen consumed during the incubation period, directly related to the amount of organic matter in the water.

**2.8.3 Total Dissolved Solids (TDS):** TDS was determined by calculating the weight difference of a beaker before and after drying the water sample. The formula used was:

$$\text{TDS (mg/L)} = (T2 - T1) / (1000)$$

*T1:* dry weight of empty beaker

*T2:* weight of the beaker after drying the sample

**2.8.4 Total Organic Carbon (TOC):** TOC was measured by injecting a sample into a TOC analyzer. The organic carbon present in the sample was oxidized to carbon dioxide, which was measured.

## 2.9 Assessment of Vegetative Growth and Biomass Accumulation

To assess the effect of dye contamination and bacterial inoculation on the growth of *Phragmites australis*, plants subjected to various treatment conditions were harvested following a 20-day experimental period.



Growth parameters such as the lengths of roots and shoots, with their fresh and dry weights, were meticulously recorded after the experiment. To ascertain dry biomass, both roots and shoots were placed in an oven maintained at 80°C and dried for 72 hours.

### 3. RESULTS

#### 3.1 Compatibility Among Bacterial Cultures

The bacterial strains LGW6 and SZ-1 were compatible, as proved by their potential to grow together on nutrient agar plates without showing significant interaction. Each bacterial strain formed separate colonies, and there were no visible inhibition zones, suggesting that both strains could coexist and grow in the presence of each other.

#### 3.2 Dye Decolorization and Degradation by Microbial Consortium

A consortium of bacterial strains SZ-1 and LGW6 effectively decolorized azo dyes in

MSM media (Figure 1). The single dye decolorization rates based on optical density for the selected bacterial strains SZ-1 and LGW6 indicate that the bacterial consortium initially decolorized the red dye (SR12) slowly but speed up its activity between days 2 and 3 incubation period. Its decolorization was high around day 3, while the decolorization rate of yellow (SR14) dye increased between days 1 and 2. The bacterial consortiums of SZ-1 and LGW6 decolorized yellow (SR14) and Red (SR12) dyes at 589 nm. The bacterial consortium decolorized 79% yellow dye and 65% red dye in single dye decolorization. The bacterial consortium efficiently degraded red and yellow dyes within four days. The optical density of the pellet was higher than the supernatant, which means that the SZ-1 and LGW6 consortium degrade SR12 and SY14 dye by the adsorption mechanism. The bacterial strain released enzymes that degraded the dye molecules and converted the toxic dye molecules into less harmful forms.



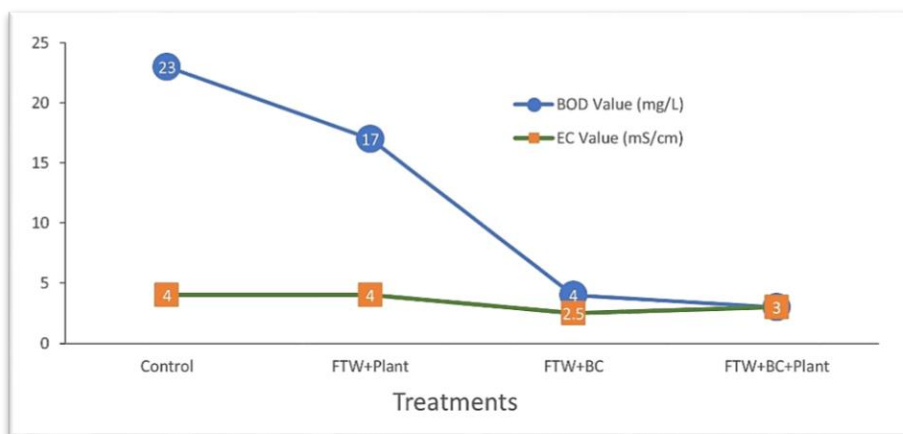
**Figure 1.** Decolorization of azo dyes by bacterial consortium LGW6 and SZ-1



### 3.3 Treatment of Textile Wastewater using Bio Augmented FTWs

In our research, FTWs carried efficient reduction in the values of electrical EC, pH, COD, BOD, and TDS of treated wastewater. Inoculated treatments with bacteria reduced physicochemical parameters more than the uninoculated treatments. Treatment 2, which contained dye, *P. australis*, and the bacterial consortium achieved good results for the EC,

TOC, BOD, COD, and TDS. The electrical conductivity (EC) of water contaminated with dye varied from 3.86 mS/cm to 2.22 mS/cm. The EC underwent minimal changes following different treatment methods. After 20 days, the EC of water in FTWs vegetated with *P. australis* (T1) recorded was at 3.12 mS/cm. In treatment T2, the EC dropped to 2.55 mS/cm, while the EC of water treated in FTWs containing bacterial consortium was 3.15 mS/cm (Figure 2).



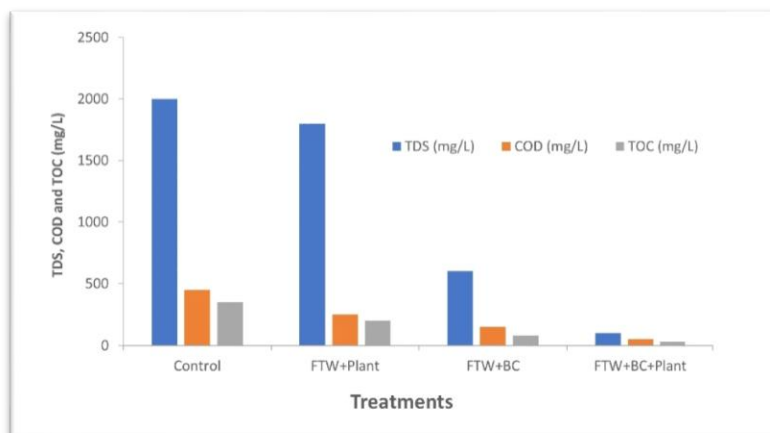
**Figure 2.** EC and BOD of textile effluent treated by FTWs

In the water treated by Floating Treatment Wetlands (FTWs) planted with *P. australis* (T1), there was an 83% decrease in total organic carbon (TOC). This reduction increased to 92% in the FTWs vegetated with *P. australis* and supplemented with a bacterial consortium (T2). A 92% reduction in TOC was observed in FTWs containing contaminated water and a bacterial consortium (T3), and in the control FTWs with contaminated water only (C), respectively.

In the FTWs containing *P. australis* (T2), a 63% decrease in BOD was observed. This decrease reached 91% in similar FTWs when supplemented with a bacterial consortium (T4), whereas the control showed only a 26% reduction. In treatment T5, BOD was reduced

by 79%. In FTWs planted with *P. australis* (T2), a 58% reduction in chemical oxygen demand (COD) was observed. This reduction increased to 94% in FTWs planted with *P. australis* and was further enhanced with a bacterial consortium (T4). Compared to the control (C), which saw a 39% decrease, treatment with the bacterial consortium led to a 62% reduction in COD. A 51% reduction in total dissolved solids (TDS) was observed in the FTWs with *P. australis* (T2). This reduction was even more significant, at 96%, in the FTWs with both *P. australis* and a bacterial consortium (T4). In comparison, FTWs with contaminated water and a bacterial consortium showed a 68% reduction in TDS, while the control noted a 30% reduction (Figure 3) and Table 1.

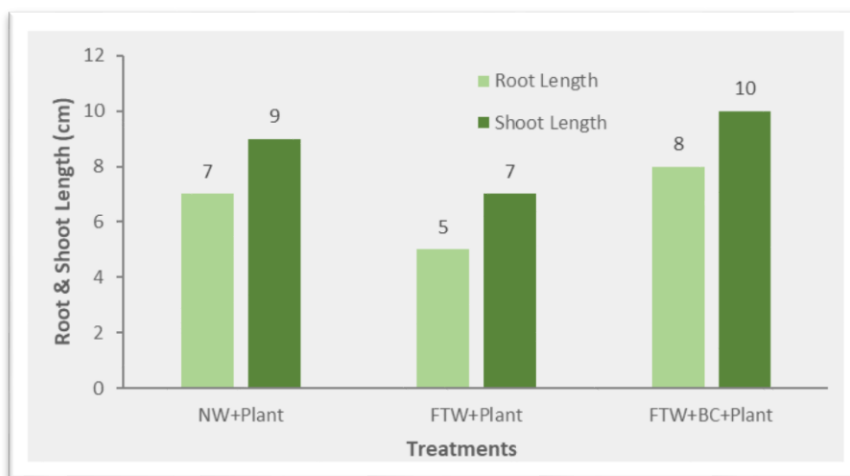




**Figure 3.** TDS, TOC and COD of textile effluent treated by FTWs

**Table 1.** Remediation of textile effluent by bacterial consortium and FTWs

Physiochemical Parameters	Before Treatment	After Treatment
pH	8.6	7.4
BOD	25 mg/L	11.9 mg/L
COD	435.42 mg/L	97.79 mg/L
TDS	1950 mg/L	1283 mg/L
EC	3.75 mS/cm	2.15mS/cm



**Figure 4.** Root and Shoot length of *P. australis* under different conditions



The augmentation of the bacterial consortium resulted in a significant decrease in EC, dropping to 2.22 mS/cm after eight days, while the untreated control sample decreased only to 3.75 mS/cm. The floating wetland treatment with the bacterial consortium showed reductions of 79%, 60%, and 66% in COD, BOD, and TDS, respectively, in the textile dye-polluted water.

### 3.4 Plant Growth and Biomass Production Assessment

The growth performance and biomass yield of *P. australis* were evaluated to determine the impacts of textile effluent exposure and bacterial inoculation on the plant's development. Exposure to textile effluent was found to suppress both growth and biomass accumulation in *P. australis*. Conversely, the introduction of beneficial bacteria was observed to enhance the plant's growth metrics significantly. Specifically, root length increased by 38%, and shoot length saw a modest improvement of 2.5% (Figure 4). Additionally, there was a notable increase in biomass production, which increased by 7.4%. This suggests that bacterial inoculation can mitigate some of the adverse effects of textile effluent on plant growth, highlighting its potential utility in phytoremediation strategies.

### 3.5 Persistence of Inoculated Bacterial Consortium

The survival and distribution of the inoculated bacterial consortium were studied in the water, shoots and roots of *P. australis*. Observations revealed that the water contained the highest number of bacteria compared to the plant parts, indicating better survival conditions in the aquatic environment. Within the plant, the roots harbored a larger population of bacteria than the shoots. This pattern suggests that while the bacteria can colonize both the plant tissues and the surrounding water, they exhibit a stronger presence or survivability in the water

and the root system than the aerial parts of *P. australis*.

## 4. DISCUSSION

Bacterial augmented FTWs play an important role in decolorizing and degrading azo dyes and decontaminating textile effluents from wastewater. For the efficient bioremediation of textile effluent, the persistence of the bacterial consortium is necessary. Vegetated FTWs inoculated with the bacterial consortium showed remarkably higher pollutant removal capability compared to unvegetated. In this research, bacterial strains LGW6 and SZ-1 showed maximum ability to decolorize dye. Both strains were compatible; therefore, their consortium was inoculated into FTWs, to examine their capacity in relationship with plants to remove pollutants (Dhameliya and Ambasana, 2023).

A large number of bacterial strains have the potential to decolorize azo dyes aerobically. The study reported that the bacterial consortium could enhance the degradation process because mixed bacterial consortia attacked the azo dye structure in various aspects. Bacteria like *Pseudomonas*, *Bacillus*, and *Escherichia coli* can decolorize azo dye using specific enzymes. In the current study, FTWs augmented with bacteria revealed greater reductions in water quality parameters such as pH, EC, COD, BOD, TDS, and TSS than the vegetated FTWs. A study reported that 8.91 was the initial pH of textile effluents and after 72 hours of treatment with *E. crassipes* and *B. cereus*, the pH was reduced to 7.90. The change in pH occurred due to the release of organic acids from the plant roots (Zahari *et al.*, 2021).

Water pollutants bind to plant roots, and nutrients uptake from water causes a decrease in EC value. TDS levels decreased due to bacterial accumulation on plant roots, where suspended particles either settled at the bottom



of FTWs or were taken up by bacteria after breakdown. COD, BOD, TDS, and TSS were removed in the reported study due to the degradation of organic pollutants by bacteria and the precipitation of suspended solids (Shen *et al.*, 2021).

Current research has shown that FTWs vegetated with *Phragmites australis* and inoculated with bacterial consortia efficiently remove textile effluents from wastewater. The synergistic approach utilized different enzymes of plants and bacteria to degrade dye, suggesting a cost-effective and eco-friendly solution for the treatment of textile wastewater. The survival and distribution of the inoculated bacterial consortium were studied in the water and in the shoots and roots of *P. australis* of treated wastewater to assess whether plant efficiency improved due to bacterial augmentation. An earlier study suggested that phytoremediation increased when bacteria were present in plant surroundings (Cangioli *et al.*, 2022).

In the present study, augmented bacteria were more abundant in wastewater with vegetative treatments than unvegetated treatments. This may be because of the presence of a symbiotic partner, leading to an increase in the abundance of augmented bacteria in wastewater with vegetation. The inoculated bacteria were taken from different parts of *P. australis*, which appeared more established in the roots and shoots of *P. australis* than the effluent. This might be because *P. australis* produces specific root exudates that interact with bacteria to facilitate their activity. Bacteria provide greater benefits to plants when colonizing plant tissues as compared to bacteria living outside the plants. A study showed that endophytic bacteria increased the uptake of pollutants and minimized phytotoxicity in their host (Alderete *et al.*, 2021).

In our study, inoculated bacteria exhibited strong persistence in the plant tissues like roots

and shoots. The water contained the highest number of bacteria compared to the plant parts, indicating a preference for better survival conditions in the aquatic environment. Early research also showed that preferential colonization of water and root systems through bacteria underscores their role in pollutant degradation and nutrient cycling within environments. To examine the impact of textile wastewater and bacterial augmentation on plant growth and activity, growth parameters like the length of root and shoot were tested. Our study showed that inoculating bacteria in textile wastewater increased the growth parameters of *P. australis*. This could be because *P. australis* has an extensive root and shoot system, which provides better oxygen to the root parts and thus helps in bacterial proliferation and breakdown of pollutants (Yadav *et al.*, 2023).

Enhanced pollutant removal in bacterial-augmented FTWs can be attributed to synergistic interactions between *P. australis* and the bacterial consortium. Bacteria likely facilitated azo dye degradation through enzyme-mediated cleavage of azo bonds (e.g., azoreductases), while plant roots provided surface area, oxygen transfer, and root exudates that supported microbial activity. Improved plant growth further contributed to pollutant uptake and stabilization within the system. From an application perspective, FTWs represent a low-cost and energy-efficient treatment option suitable for decentralized wastewater management. The use of locally available plants and indigenous bacteria enhances feasibility and scalability, particularly in developing regions.

FTWs are a sustainable and environmentally friendly approach to treating industrial wastewater. They are easy to construct and operate, making them feasible for developing nations. The study proved that the cost of using constructed wetlands to remove pollutants



from wastewater is 50-90% lower than conventional wastewater treatment methods.

## 5. Conclusion

Bacterial augmentation significantly improves the performance of *Phragmites australis*-based floating treatment wetlands for the remediation of azo dye-contaminated textile effluent. The synergistic plant-microbe system enhanced pollutant removal, reduced phytotoxic effects, and promoted plant growth. These findings support the potential application of bacterial-augmented FTWs as a sustainable, eco-friendly, and economically viable wastewater treatment technology. Further studies focusing on molecular characterization of bacterial strains and pilot-scale implementation are recommended.

### Conflict of interest

The authors declare no conflict of interest.

### Author Contributions

MAA and SS conducted the experiments and prepared the draft, HZW and IA analyzed the results and edited the draft, IA and KF supervised the research and finalized the draft.

### Ethics Approval and Informed Consent

Not applicable

### Availability of Data

Not applicable

### Funding

Not applicable

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