Effectiveness of Endophytes Bacteria in Enhancing Floating Treatment Wetland to Treat Textile Wastewater

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ABSTRACT
This research investigated the association of consortium endophyte bacteria from different hosts to enhance the performance of *Vetiveria zizanioides* in treating textile wastewater using Floating Treatment Wetlands (FTWs). The endophyte bacteria were isolated from the roots of three natural plants (*Oryza sativa*, *Colocasia esculenta*, and *Alternanthera philoxeroides*) contaminated by textile wastewater. The selected isolated endophyte bacteria were subjected to the four FTWs reactors containing the *Vetiveria* sp. and ran for 30 days in a semi-batch system to evaluate their performance. FTWs reactors-augmented endophyte bacteria could reduce the COD, color, and heavy metals in textile wastewater. The highest removal efficiencies of COD (74%) and color (91%) were observed in FK2 (vegetated control) and F4 reactor, respectively. The addition of endophyte bacteria increased the heavy metal reductions of Pb (52%) and Cd (33%) in reactors of F3 and F4, respectively. This study exhibited that the consortium endophyte bacteria isolated from the contaminated plants could improve the FTWs reactor performance. Finally, they reduce the plant stresses in the contaminated wastewater by increasing the plant biomass in roots and shoots. These findings reveal that the consortium of natural endophyte bacteria from different hosts does not inhibit their function and association with the other host plant, but they contribute positive responses to the plant growth and pollutant degradation.

Keywords: bioaugmentation, consortium endophyte bacteria, Phytoremediation, *Vetiveria zizanioides*.

INTRODUCTION
Textile wastewater poses several significant environmental issues, particularly from small and medium enterprises (SMEs) that produce traditional Indonesian woven fabrics. The primary problem of SMEs textile industries is the generation of wastewater during the dyeing process and introducing the wastewater into the environment without any treatment. Textile wastewater typically contains high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), lead (Pb), and color (Kiswanto et al., 2019; Rahmadyanti & Febriyanti, 2020; Suhartini et al., 2019). Azo dyes are the most common synthetic dyes used in the textile industry because they are easy to get, low-cost, stable, and have a wide range of colors (Saratale et al., 2011; Singh et al., 2014). The presence of dyes in water sources negatively impacts human health, increasing the mutagenic and carcinogenic characteristics in the body, contributing to environmental pollution and disrupting the aquatic ecosystem by reducing dissolved oxygen through sunlight obstruction in water sources (Samsami et al., 2020; Sudarshan et al., 2023).
Several physicochemical treatments such as electrochemical oxidation (Nhan & Luu, 2023), adsorption (Adegoke & Bello, 2015), coagulation-flocculation (Daud et al., 2023), and direct contact membrane distillation (Fortunato et al., 2021) have sufficiently removed the pollutants in textile wastewater. However, those techniques require high operational costs, engineering skills, high energy, and chemical demands (Crini & Lichfouse, 2019; Moyo et al., 2022). On the other hand, eco-friendly methods and low-cost technology like wetlands have been widely applied to treat various contaminated wastewater, including domestic (Gusti Wibowo et al., 2023) and industrial wastewater (Ashraf et al., 2018; Azanaw et al., 2022). Floating treatment wetlands (FTWs) are one of the technologies in wetland treatments that have been used for remediating textile wastewater as a low-cost conventional and sustainable wastewater treatment approach (Darajeh et al., 2019; Wei et al., 2020). FTWs use a phytoremediation mechanism, utilizing vegetation on a floating mat to uptake pollutants, produce biomass, and facilitate phytodegradation (Kafle et al., 2022). Plant roots play a significant role in FTWs system by aiding nutrient uptake, providing surface area for biofilm attachment, filtration/adsorption mechanism, and producing enzymes (Shahid et al., 2020; Wei et al., 2020). The selection of plant types for phytoremediation is an important role to enhance the wetland performance in degrading the wastewater. *Vetiveria zizanioides* is a suitable criteria for a phytoremediation plant because it provides large phytomass and robust massive roots systems that promote an effective remediation by degrading the pollutants (Badejo et al., 2018; Kiamarsi et al., 2020). *Vetiveria sp* has been reported in the high removal of physico-chemical contaminants in domestic wastewater (Badejo et al., 2018), hydrocarbon remediation (Kiamarsi et al., 2020), and textile wastewater (Tambunan et al., 2018). However, FTWs using *Vetiveria sp* have limitations, including a lack of consistency, ineffective purification performance, and low degradation in toxic conditions (Tara et al., 2019a).

To address this limitation, the addition of plant growth-promoting (PGP) bacteria, such as endophyte bacteria, has been recommended to leverage their capability to degrade pollutants and enhance plant growth. Endophyte bacteria communities mostly live in the soils near plant roots or interior tissue of roots (Tara et al., 2019a), supporting plant growth and creating conditions for plants to survive in the toxic contaminated media (Ijaz et al., 2016). The inoculant of endophyte bacteria increases nutrient uptake for plant growth and biomass (Li et al., 2023), reduces the plant stresses (Nanda et al., 2019), controls metal resistance mechanisms, and either encourages or inhibits the accumulation of heavy metals in plants by phytoextraction and phytostabilization (Begum et al., 2018; Fan et al., 2018). However, the endophyte bacterial communities are influenced by the host plant species, type of soils, and environmental conditions (Ding & Melcher, 2016). This condition alters the plant mechanisms in the contaminated wastewater, particularly in responding to pollutants degradation and root stresses during phytoremediation.

Therefore, the research purpose of this study was to evaluate the effectiveness of consortium endophyte bacteria from different plant hosts in enhancing the performance of *Vetiveria zizanioides* in treating textile wastewater in the system of floating treatment wetlands. The isolations of endophyte bacteria from natural plants contaminated wastewater encourage a chance to enhance the FTWs in treating textile wastewater. The colonized bacteria in different groups of plant might introduce various benefits or inhibits in providing nutrition, protecting the plant from toxic conditions, and augmenting the heavy metals in plant tissues. This research’s findings can propose a different point of view for textile home industries to conduct the textile wastewater treatment with eco-friendly technology.

**MATERIALS AND METHODS**

**Isolation and identification of endophytic bacteria**

Endophytic bacteria were isolated from three different plant roots contaminated textile wastewater (*Oryza sativa, Colocasia esculenta*, and *Alternanthera philoxeroides*). The plant samples were collected from healthy and mature plants that received textile wastewater from local home industry effluents at the drainage channel. The roots and shoots samples were placed in ziploc plastic bags, stored in an ice box during transportation to the laboratory, and stored in a refrigerator at 5°C before lab analysis the next day. The endophytic bacteria were isolated from
surface-sterilized plant roots by following the instructions of Shehzadi et al. 2016 with a minor modification. Root samples underwent several steps, including a brief 2-minute wash in sterile-distilled water, a minute sterilization with 70% ethanol, 15 minutes of immersion in 1.2% NaClO solution, and three subsequent washes with sterile distilled water by shaking (15 minutes each). Surface sterilized roots (1 g) were homogenized with 20 ml of 0.9% NaCl solution. Then, 300 µl suspensions were spread onto nutrient agar containing 7% filter-sterilized textile wastewater and incubated at 30℃ for 48 hours. Identification of inoculants involved analyzing their morphology, including shape, edge/margin, elevation, opacity, and pigment, using a microscope referred to the American Type Culture Collection (ATCC). The selected inoculants with different morphology were incubated in 50 ml nutrient broth at 30℃ for 24 hours. The inoculant bacteria were stored in the refrigerator prior to being used in the floating wetland treatments within three days.

Experimental set up of floating treatment wetland

The floating treatment wetlands were conducted in a 5 L bucket with a dimension of diameter of 18 cm and a height of 19 cm (Fig. 1a). The Vetiveria zizanioides shoots were cut to a length of 15 cm above the roots and were planted to FTWs that contained two pots. Each pot consisted of three stem plants and three layers of media 1.5 cm coconut fibers, 1.5 cm sands, and 5 cm soils. The six different FTWs were prepared for control textile wastewater only (FK1), control textile wastewater and plant (FK2), treatments of textile wastewater-plant and consortium bacteria from Oryza sativa (F1), Colocasia esculenta (F2), Alternanthera philoxeroides (F3), and mix all consortium bacteria (F4). Then, the FTWs reactor was acclimatized in tap water for 30 days and a small amount of hydroponic fertilizer (containing 2% P₂O₅; 0.10% Fe; 0.10% vitamin B1; 0.04% NAA) was added to each FTWs once a week to improve the plant growth.
Textile effluent degradation

After the acclimatization period, the water in FTWs was replaced with 25% dilution of textile wastewater and 10 mL of inoculant consortium bacteria were added. The FTWs operated for 30 days, while monitoring water quality for physicochemical parameters of pH, TDS, EC, temperature, COD, TSS, color, and heavy metals (Cadmium – Cd and Lead – Pb) to observe their performance. The physicochemical parameters were analyzed following the water and wastewater Indonesian National Standard (Environmental and Forestry Instrument Standardization Agency (BSILHK), 2023), referring to Standard Methods for the Examination of Water and Wastewater, APHA. Due to the dry season, there was a significant reduction in wastewater volumes, which affected the addition of fresh textile wastewater after the sampling collection. The sampling times and volume of wastewater used in the FTWs are shown in Table 1.

Biomass plant growth

The shoots height and roots length of plant biomass were measured before treatment as an initial condition. The shoot and root samples were harvested after the treatment from the FTWs, and they were thoroughly cleaned from soils, water, and other attached particles. The roots and shoots plant biomass were dried at 70°C for 48 hours and measured to obtain dry weight.

Statistical analysis

The statistical analysis uses IBM SPSS Statistics 25 to identify the significant difference ($p < 0.05$). The significant differences between the days and treatments were analyzed with ANOVA, and the Duncan test to determine which treatments possess distinctions.

RESULTS AND DISCUSSION

Textile wastewater characteristics

Physico-chemical characteristics of textile wastewater from effluents of textile industries used in this study are shown in Table 2. The effluent concentrations after dying processes exhibited high concentrations in COD (3,750 mg/L), color (3,514 mg/L) and pH of 11. These concentrations exceeded the National Industrial Wastewater Standard (NIWS) for textile wastewater set by the Industrial Ministry of Indonesia. The effluent from home industries of textile dying process is introduced to the environment directly without prior treatment. The high concentrations of textile

| Table 1. Sampling time and additional textile wastewater to the FTWs reactors |
|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Days | 0 | 5 | 8 | 10 | 16 | 23 | 30 |
| Parameter test/ sampling | √ | √ | √ | √ | √ | √ | √ |
| Refill textile wastewater in reactor | √ | √ | √ | √ | √ | √ | √ |
| The amount of refill (ml) | 2000 | 250 | 400 | 450 | 250 |

| Table 2. Textile wastewater quality discharge from the local home industry located in East Java Province |
|-------------------|----------------|----------------|----------------|
| Parameter | Reactor FK1, F1, F2, F3, F4 | Reactor FK2 | NIWS* |
| COD (mg/L) | 3,750 | 1,937.50 | 150 |
| TSS (mg/L) | 180 | 480 | 50 |
| Color (unit Pt-Co) | 3,514.29 | 3,442.86 | 200 |
| EC (µS/cm) | 1,466 | 3,550 | NG |
| TDS (mg/L) | 1,620 | 2,180 | NG |
| Temperature (°C) | 27 | 26.40 | NG |
| pH | 11 | 10.40 | 6 – 9 |
| Cd (mg/L) | 0.007 | NM | NG |
| Pb (mg/L) | 0.177 | NM | NG |

Note: National Industrial Wastewater Standard (NIWS) for textile wastewater set by Industrial Ministry of Indonesia, regulation of LHK No. 5/2015.
wastewater pose a danger to the aquatic environment and organisms and shrink the natural water quality (Al-Tohamy et al., 2022). Therefore, textile wastewater is required to be treated before discharge into the environment.

**Characteristics of endophyte bacteria**

This study isolated endophyte bacteria from the roots of three natural plants (*Oryza sativa, Colocasia esculenta, and Alternanthera philoxeroides*) contaminated with textile wastewater. The isolated bacteria from three natural plants were evaluated for morphology, and bacteria with differences in characteristics and dominant number in the colony were selected. The number of selected colonies from each natural plant are displayed in Table 3. Then, the chosen consortium bacteria were inoculated and added to each FTWs reactor.

**Performance of FTWs reactor**

**Physical parameter**

The performance of physical parameters along treatments is presented in Table 4. The physical concentrations fluctuated during the treatment process due to the addition of textile wastewater. The levels of TSS, TDS, EC, temperature, and pH between FK2 (vegetated control) and FTWs showed a significant difference ($p < 0.05$). The pH values decreased from 11 to neutral pH. While all of FTWs reactors showed high levels of TDS and EC, their levels were not significantly different through the 30 days of treatment. The high level of TDS and EC in the textile wastewater is due to the dye processes utilizing synthetic azo dyes and other chemicals that affect the ionic release to the wastewater.

### Table 3. Selected colony morphology of endophyte bacteria from natural treatment-contaminated textile wastewater

<table>
<thead>
<tr>
<th>Contaminated plant samples</th>
<th>Type of colony</th>
<th>FTWs Reactor</th>
<th>Colony morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>F1</td>
<td>circular, convex</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td>R1 c&lt;sub&gt;2&lt;/sub&gt;</td>
<td>filamentous</td>
<td>opaque, white</td>
</tr>
<tr>
<td></td>
<td>R1 c&lt;sub&gt;3&lt;/sub&gt;</td>
<td>irregular</td>
<td>raised, opaque</td>
</tr>
<tr>
<td><em>Colocasia esculenta</em></td>
<td>R2 a</td>
<td>F2</td>
<td>circular, raised</td>
</tr>
<tr>
<td></td>
<td>R4 a&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td>opaque, creamy yellow</td>
</tr>
<tr>
<td><em>Alternanthera philoxeroides</em></td>
<td>R4 a&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>filamentous, flat</td>
</tr>
<tr>
<td></td>
<td>R4 a&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td>opaque, white</td>
</tr>
<tr>
<td></td>
<td>R4 a&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td>irregular, raised</td>
</tr>
</tbody>
</table>

### Table 4. Degradation of the textile wastewater by FTWs reactors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>FK1</th>
<th>FK2</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>2322.92&lt;sup&gt;a&lt;/sup&gt; (490.11)</td>
<td>1203.13&lt;sup&gt;b&lt;/sup&gt; (235.76)</td>
<td>2322.92&lt;sup&gt;a&lt;/sup&gt; (521.52)</td>
<td>3218.75&lt;sup&gt;b&lt;/sup&gt; (777.91)</td>
<td>2145.83&lt;sup&gt;a&lt;/sup&gt; (411.32)</td>
<td>2125&lt;sup&gt;a&lt;/sup&gt; (384.26)</td>
<td></td>
</tr>
<tr>
<td>Color (Pt-Co)</td>
<td>4692.86&lt;sup&gt;a&lt;/sup&gt; (253.38)</td>
<td>1015.24&lt;sup&gt;c&lt;/sup&gt; (514.59)</td>
<td>2688.33&lt;sup&gt;b&lt;/sup&gt; (588.41)</td>
<td>3487.14&lt;sup&gt;b&lt;/sup&gt; (914.87)</td>
<td>3205&lt;sup&gt;a&lt;/sup&gt; (686.57)</td>
<td>2541.90&lt;sup&gt;a&lt;/sup&gt; (550.12)</td>
<td></td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>266.67&lt;sup&gt;a&lt;/sup&gt; (58.18)</td>
<td>365&lt;sup&gt;a&lt;/sup&gt; (92.62)</td>
<td>231.67&lt;sup&gt;a&lt;/sup&gt; (53.50)</td>
<td>228.33&lt;sup&gt;a&lt;/sup&gt; (45.34)</td>
<td>290&lt;sup&gt;a&lt;/sup&gt; (40.25)</td>
<td>190&lt;sup&gt;a&lt;/sup&gt; (47.40)</td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>3715.38&lt;sup&gt;a&lt;/sup&gt; (163.41)</td>
<td>2349.5&lt;sup&gt;b&lt;/sup&gt; (152.66)</td>
<td>3573.94&lt;sup&gt;a&lt;/sup&gt; (319.09)</td>
<td>3402.63&lt;sup&gt;a&lt;/sup&gt; (341.78)</td>
<td>3399.75&lt;sup&gt;a&lt;/sup&gt; (336.75)</td>
<td>3687.38&lt;sup&gt;a&lt;/sup&gt; (306.27)</td>
<td></td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>2810.63&lt;sup&gt;a&lt;/sup&gt; (119.07)</td>
<td>1804.06&lt;sup&gt;a&lt;/sup&gt; (64.48)</td>
<td>2629.38&lt;sup&gt;a&lt;/sup&gt; (231.53)</td>
<td>2551.88&lt;sup&gt;a&lt;/sup&gt; (223.62)</td>
<td>2482.56&lt;sup&gt;a&lt;/sup&gt; (256.89)</td>
<td>2775.63&lt;sup&gt;a&lt;/sup&gt; (228.04)</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.87&lt;sup&gt;b&lt;/sup&gt; (0.27)</td>
<td>28.9&lt;sup&gt;a&lt;/sup&gt; (0.47)</td>
<td>26.34&lt;sup&gt;a&lt;/sup&gt; (0.24)</td>
<td>26.38&lt;sup&gt;a&lt;/sup&gt; (0.26)</td>
<td>26.58&lt;sup&gt;a&lt;/sup&gt; (0.29)</td>
<td>26.54&lt;sup&gt;a&lt;/sup&gt; (0.27)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>8.72&lt;sup&gt;a&lt;/sup&gt; (0.36)</td>
<td>7.26&lt;sup&gt;a&lt;/sup&gt; (0.30)</td>
<td>8.05&lt;sup&gt;a&lt;/sup&gt; (0.32)</td>
<td>7.92&lt;sup&gt;a&lt;/sup&gt; (0.33)</td>
<td>8.04&lt;sup&gt;a&lt;/sup&gt; (0.31)</td>
<td>7.67&lt;sup&gt;a&lt;/sup&gt; (0.36)</td>
<td></td>
</tr>
<tr>
<td>Cd (mg/L)</td>
<td>0.008&lt;sup&gt;a&lt;/sup&gt; (0.001)</td>
<td>-</td>
<td>0.009&lt;sup&gt;a&lt;/sup&gt; (0.001)</td>
<td>0.010&lt;sup&gt;a&lt;/sup&gt; (0.001)</td>
<td>0.010&lt;sup&gt;a&lt;/sup&gt; (0.001)</td>
<td>0.008&lt;sup&gt;a&lt;/sup&gt; (0.001)</td>
<td></td>
</tr>
<tr>
<td>Pb (mg/L)</td>
<td>0.176&lt;sup&gt;a&lt;/sup&gt; (0.011)</td>
<td>-</td>
<td>0.147&lt;sup&gt;a&lt;/sup&gt; (0.019)</td>
<td>0.148&lt;sup&gt;a&lt;/sup&gt; (0.023)</td>
<td>0.165&lt;sup&gt;a&lt;/sup&gt; (0.016)</td>
<td>0.160&lt;sup&gt;a&lt;/sup&gt; (0.023)</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** each value is the mean of six data from weekly sampling. The values in parenthesis indicate standard error. Means in the same rows followed by the same letter are not significantly different.
**Chemical degradations**

The concentrations of COD and color fluctuated in all of FTWs reactors, particularly after the addition of fresh wastewater treatment (Fig. 2). Significant difference between FK1 (unvegetated control) and other FTWs reactors did not display significant differences between treatments despite the addition of volume wastewater introduced after sampling. The FK1 exhibited a reduction in COD concentration (removal 53%) and no additional wastewater along treatment. The FK1 showed a decline in COD concentrations, possibly due to the influence of living organisms and remaining oxygen in the wastewater. On the contrary, in the first five days of observation, F2 reactor showed an increase in COD concentration compared to other FTWs reactors, which it is due to the stresses of plant roots in the circumstance of polluted wastewater (Adeleke et al., 2021), releases the soluble organic from plant tissue to adapted with the environment (Benny & Chakraborty, 2023), and the undergo of consortium bacteria in the plant tissue (Sun et al., 2021).

The highest efficiency removal was identified in FK2 (74%) compared to other FTWs after 30 days of treatment. In comparison, the FTWs reactors, with the addition of the consortium bacteria, exhibited a COD reduction of around 70 – 73% compared to the FK1. A significant difference ($p < 0.05$) in COD concentrations was observed between FK2 and F2; however, the comparison between FTWs reactors with the addition of a consortium bacteria did not show a significant difference ($p > 0.05$). This finding indicates that *Vetiveria* sp. can reduce COD concentration from textile wastewater without consortium bacteria.

![Fig. 2. Degradation of COD (a) and color (b) in FTWs. The different treatments were FK1 (control textile wastewater only), FK2 (control textile wastewater and plant), F1 (textile wastewater and consortium bacteria from *Oryza sativa*), F2 (textile wastewater and consortium bacteria from *Colocasia esculenta*), F3 (textile wastewater and consortium bacteria from *Alternanthera philoxeroides*), and F4 (textile wastewater and mix all of consortium bacteria). Each value is the mean of two replicates, the error bar represents the standard error, and means followed by the same letter are not significantly different at a 5% significance level](image)
Several researchers have reported that *Vetiveria sp* could degrade various wastewater, including textile wastewater, domestic, industrial, and hydrocarbon sources (Badejo et al., 2018; Kiamarsi et al., 2020; Najam-Us-Sahar et al., 2017). In the phytoremediation process, plants conduct many mechanisms to reduce the pollutants, such as degradation (rhizo-degradation, phytodegradation), accumulation (phytoextraction, rhizofiltration), dissipation (phytovolatilization), and immobilization the pollutants (Pivets, 2001). These mechanisms are affected by pH, electric conductivity, organic contaminants, microbial processes, soil amendments, and toxic pollutants (Guidi Nissim et al., 2018). Moreover, the augmentation of endophyte bacteria in FTWs reactors enhanced the pollutant reduction compared to the reactor without the addition of endophyte bacteria (Rehman et al., 2018). This study revealed that different host plants of endophyte bacteria could assimilate with another plant in the contaminated textile wastewater. The isolated consortium endophyte bacteria contributed a reduction of organic compounds, which showed the endophyte bacteria breakdown the organic compounds and utilized them for their metabolite and plant nutrition source (Yousaf et al., 2010). This reduction is likely attributed to the combined enzymatic activity of bacteria and plants converting organic matter into simpler metabolites. Then, plants can easily up-take the organic pollutants as part of the nutrient assimilation process or remove them from the system in gaseous form, such as CO₂ and N₂ (Tara et al., 2019b).

The color degradations were significant differences (p < 0.05) in all of FTWs reactors with the consortium bacteria isolated from natural plants compared to FK1 (Fig. 3). While the significant differences between FTWs with the addition of bacteria and between the sampling days were insignificant (p > 0.05). The higher color reduction was achieved in the F4 reactor (97% removal) with the addition of isolated bacteria from three plant hosts. A high decolourization removal (91%) was also identified in the FK2 (vegetated control) compared to the FK1. All of the FTWs reactors exhibited a decrease in color concentrations, with a greater efficiency achieved by the plant inoculated with endophyte bacteria. The combination of endophytes bacteria associated with *Vetiveria sp* has demonstrated outstanding potential for color removal (Badejo et al., 2018). This result aligns with previous studies attributing enhanced efficiency to the enzymatic activity of plants and bacteria converting color into simple metabolites (Tara et al., 2019b). Chandanshive et al., 2016 emphasized that the main factor enhancing dye degradation is the contribution of the dye-degrading enzyme produced by plant roots in extreme conditions, which helps the plant reduce plant stresses.

### Heavy metal degradation and its accumulation

The heavy metals concentrations in treated textile wastewater are displayed in Figure 3. The concentrations of Pb and Cd in the FK1 were consistent during the treatment, and these heavy metal concentrations fluctuated during the treatment due to the addition of fresh textile wastewater in small volumes after sample collection. In the effluent, the F4 reactor showed the lowest concentration of Cd (p < 0.05), while the concentration of Pb was detected low in the F3 reactor compared to other treatments (p < 0.05). The removals of Cd and Pb in both reactors were 33% and 52%, respectively. These removals indicate the adsorption process by the *Vetiveria* sp. using the roots and transportation of heavy metals into the tissue of shoot plants.

The accumulations of heavy metals in the roots and shoots of *Vetiveria zizanioides* are presented in Table 5. The roots and shoots of *Vetiveria* sp. plants in FK2 reactor (vegetated control) exhibited the lowest uptake of Cd and Pb. On the other hand, the heavy metals were detected more accumulated in the roots and shoots of plants in the FTWs reactor with the addition of consortium bacteria compared to the FK1. This finding revealed the accumulation of heavy metals by *Vetiveria sp* without addition of isolated endophyte bacteria (FK2) much lower than in FTWs reactors isolated bacteria. The F1 reactor added the consortium bacteria isolated from *Oryza sativa* containing high Pb and Cd accumulations in the roots and shoots. The accumulations of Pb were primarily stored in the plant shoot tissue rather than in the roots, while the Cd detected slightly more accumulated in the roots tissue than in the shoot. Based on these results, *Vetiveria zizanioides* plant can accumulate heavy metals in tissues of roots and shoots. The addition of natural endophyte bacteria could increase the plant uptake of heavy metal pollutants.

In this study, the accumulation of heavy metals (Cd and Pb) could be confirmed in the plant tissue of roots and shoots of *Vetiveria zizanioides,*
Fig. 3. Degradation of Cd (a) and Pb (b) in the effluent of FTWs reactor treating textile wastewater. The heavy metal concentrations in FK2 were not presented in this study. The different treatments were: FK1 (control textile wastewater only), F1 (textile wastewater and consortium bacteria from *Oryza sativa*), F2 (textile wastewater and consortium bacteria from *Colocasia esculenta*), F3 (textile wastewater and consortium bacteria from *Alternanthera philoxeroides*), and F4 (textile wastewater and mix all of consortium bacteria). Each value is the mean of two replicates, the error bar represents the standard error, and means followed by the same letter are not significantly different at a 5% significance level and the uptake of the heavy metals increased in FTWs reactors with the natural endophytes bacteria. Similar findings indicated that the addition of endophyte bacteria to the host plant improved the removal of heavy metals in the textile wastewater (Hussain et al., 2018). The mechanism of endophyte bacteria to remediate heavy metals varies and is close to its mutualistic relationship with the hosts. Moreover, the presence of inoculant augmenting bacteria in the roots plays an important role to prevent the host stress caused by biotic and abiotic circumstances such as salinity, high-temperature stressors, drought, pathogen infection, and toxic contaminants in contaminated areas mainly containing heavy metals (Nanda et al., 2019; Sharma & Kumar, 2021). Generally, endophytes colonize the root surface of plants and get into the tissue while maintaining their own life with nutrients available in the surrounding environment (Afzal et al., 2019; Hardoim et al., 2008; Prakash, 2021). The colonization of endophytes attached to the root enables the synthesis of growth hormones in the plants, thus enhancing plants ability to reduce heavy metals (Etesami et al., 2015). The removals of heavy metals by endophyte bacteria differ based on the order of Fe > Cr > Mn > Ni > Zn > Cd > Cu at pH normal, which affects the different transport of heavy metals in the plant tissues (Nayak et al., 2018). The efficiency of heavy metal removal and uptake increases by the presence of *Bacillus* sp. to reduce uranium, lead, copper, hexavalent chromium, and
other ion metals from the contaminated media (Zhuang et al., 2011).

**Plant biomass and growth**

The biomass of *Vetiveria zizanioides* plants was harvested after 30 days of treatments and measured for roots and shoots weight (Figure 4). The fresh shoot weights of FK2 and F4 were heavier than the biomass of other FTWs reactors, weighing 4.9 g and 4.1 g, respectively. And, the F2 and F4, with the addition of consortium bacteria, in fresh and dry root weight exhibited heavier biomass compared to other FTWs reactors. However, the biomasses of dry roots showed no significant differences between the F2 and F4 compared to the FK2 FTW reactor \( p < 0.05 \). These findings indicate some consortium endophyte bacteria from natural plants could symbiosis with the roots of *Vetiveria zizanioides*. It positively affected the plant’s ability to survive in the toxic textile wastewater and showed the ability to grow under stress conditions. Plant roots with the addition of consortium bacteria showed a higher weight than roots without a bacterial inoculant (FK2). *Vetiveria* sp. plants of FK2 showed a greater number of yellowish or dry leaves, and a plant was found dead at the end of treatment compared to the plants in the FTWs reactors inoculated with consortium bacteria. These

**Table 5.** Heavy metal accumulation in *Vetiveria sp* plants in treated textile wastewater

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root (mg/kg)</th>
<th>Shoot (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd</td>
<td>Pb</td>
</tr>
<tr>
<td>FK2</td>
<td>1.42 (0.35)*</td>
<td>81.32 (9.25)*</td>
</tr>
<tr>
<td>F1</td>
<td>3.98 (1.15)*</td>
<td>102.12 (25.43)*</td>
</tr>
<tr>
<td>F2</td>
<td>2.35 (0.84)*</td>
<td>47.80 (19.65)*</td>
</tr>
<tr>
<td>F3</td>
<td>3.10 (1.15)*</td>
<td>95.19 (34.67)*</td>
</tr>
<tr>
<td>F4</td>
<td>3.54 (0.18)*</td>
<td>98.65 (5.78)*</td>
</tr>
</tbody>
</table>

Note: Each value is the mean of two replicates with six measurements. The different treatments were: FK1 (control textile wastewater only), F1 (textile wastewater and consortium bacteria from *Oryza sativa*), F2 (textile wastewater and consortium bacteria from *Colocasia esculenta*), F3 (textile wastewater and consortium bacteria from *Alternanthera philoxeroides*), and F4 (textile wastewater and mix all of consortium bacteria). Each The values in parenthesis indicate standard error. Means in the same rows followed by the same letter are not significantly different. LD means limit of detection.

![Fig. 4. The roots and shoots plan biomass of FTWs reactor after 30 days of treatment. The different treatments were: FK1 (control textile wastewater only), F1 (textile wastewater and consortium bacteria from *Oryza sativa*), F2 (textile wastewater and consortium bacteria from *Colocasia esculenta*), F3 (textile wastewater and consortium bacteria from *Alternanthera philoxeroides*), and F4 (textile wastewater and mix all of consortium bacteria). Each value is the mean of six biomasses of roots and shoots plant. The values in parenthesis indicate standard error. Means in the same rows followed by the same letter are not significantly different.](image-url)
results emphasize that plants maintain complex ecosystems, and the endophyte bacteria interact and compete for nutrients and water in the rhizosphere and endosphere of the host plant (Ye et al., 2016). Moreover, many studies have revealed that endophyte bacteria isolated from roots and shoots can improve plant health and growth in a contaminated environment through several mechanisms involving mineralization of organic pollutants, production of growth hormones and stress relieving hormones, and increased water absorption and mineral nutrition (Ashraf et al., 2018).

CONCLUSIONS

This study revealed positive benefits on the effectiveness of consortium bacteria from different plant hosts to improve the Vetiveria zizanioides plant in reducing the pollutants of textile wastewater. Vetiveria zizanioides plant (FK2-vegetate control reactor) could reduce COD and color in textile wastewater with a 74% and 91% removal percentage, respectively. The association of augmented endophyte bacteria from different host plants showed positive response with Vetiveria sp. by reducing organic and inorganic compounds such as color (97%) and Pb (52%) in the F4 and F3, respectively. The accumulations of Cd and Pb could be confirmed in the roots and shoots tissues of Vetiveria zizanioides. The accumulation of heavy metals increased in FTWs reactors with the addition of isolated endophyte bacteria compared to Vetiveria sp. plant only. And the plant associated with augmented endophyte bacteria improved of plant biomass in the roots and shoots. These results concluded that the isolated endophyte bacteria from different hosts could be applied in the other plants to treat the textile wastewater. The association between them was confirmed in a positive response to improve plant growth and degrade the pollutants in textile wastewater. Further research on enhancing performance and combining with sustainable eco-green technology will be considered to accomplish the effluent standard and improve pollutant degradation.

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