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Research article

Integrated perspectives on the use of bacterial endophytes in horizontal flow constructed wetlands for the treatment of liquid textile effluent: Phytoremediation advances in the field

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ABSTRACT

Constructed wetlands (CWs) have emerged as cost-effective and sustainable treatment systems for the remediation of industrial wastewaters; nevertheless, their potential has mostly been evaluated in laboratory-scale studies. Likewise, endophytic bacteria can enhance plant growth and reduce phytotoxicity under polluted conditions, but their application with pilot-scale CWs has rarely been evaluated. The present study aims to evaluate on-site performance of endophyte-assisted pilot-scale horizontal flow constructed wetlands (HFCWs) for the remediation of effluent from a textile industry. The HFCWs were established by planting *Leptochloa fusca* in the presence of three endophytic bacterial strains with dye degrading, and plant growth promoting capabilities. We found that the system was able to remove a significant proportion of both organic and inorganic pollutants. Maximum reduction of pollutants was observed in endophyte-augmented HFCWs, where the COD and BOD reduced from 493 to 70 $mg\,l^{-1}$ and 190 to 42 $mg\,l^{-1}$, respectively, within 48 h. Additionally, survival of endophytic bacteria in different components of the HFCWs was also recorded. Treated wastewater was found to be non-toxic and the inoculated bacteria showed persistence in the wastewater as well as rhizo- and endosphere of *L. fusca*. Accordingly, a positive impact on plant growth was observed in the presence of bacterial augmentation. The system performance was comparable to the vertical flow constructed wetlands (VFCWs) as high nutrients reduction was seen in the presence of this partnership. This pilot-scale study is a step forward toward the field-scale application of phytoremediation coupled with bacterial endophytes as a cost-effective means of on-site wastewater remediation. To the best of our knowledge, this is among the first pilot-scale studies on use of HFCWs for improvement in quality of textile industry effluent as most previous studies are limited either in the context of engineering or lack effective interplay of plant and bacteria.

1. Introduction

Industrial effluents comprise of a variety of pollutants that pose serious threats to the quality of ecosystems that receive them during disposal. Wastewater produced during fabric production is considered to be one of the most polluted industrial effluents due to the presence of numerous chemical compounds including dyes, detergents, pigments, salts, heavy metals, sulfates, chlorides, oil, and grease, etc. With their carcinogenic and mutagenic nature, these chemicals are toxic to both fauna and flora that are exposed to their elevated concentrations (Bafana et al., 2009, 2011). Moreover, the presence of dyes and pigments in the effluent decreases light penetration in the receiving water bodies that ultimately decreases photosynthetic activity, and thus, dissolved oxygen concentrations in them (Pearce et al., 2003; Sarayu and Sandhya, 2012; Waranusantigul et al., 2003).

Constructed wetlands (CWs) are artificial systems designed to treat variety of industrial wastewaters. Low capital investment and minimum operational costs make the technology feasible and applicable for the

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remediation of sewage and industrial wastewater in developing countries (Vymazal, 2009; Vymazal and Kröpfelová, 2009; Wu et al., 2014). In a typical CW system, plants are vegetated in a permeable medium composed of soil, sand, and gravel that allows wastewater to percolate through itself (Davies et al., 2006; Kabra et al., 2013; Rane et al., 2016; Zhang et al., 2018) while establishing a direct contact with plant roots and microbial biofilm growing on the roots and surface of submerged CW components (Schröder et al., 2008). This interaction results in the removal of a wide range of pollutants from wastewater due to the diverse pollutant uptake and degradation mechanisms that can take place within plants and their associated microbial communities (Afzal et al., 2014; Khandare et al., 2013b; Weyens et al., 2009). Plants carry out physical entrapment (filtration) of nutrients and heavy metals through their extensive root systems (Alemu et al., 2017; Bharathiraja et al., 2018; Hussein and Scholz, 2018), while the endophytic bacteria that colonize their root and shoot interiors play a key role in the degradation of pollutants that translocate to aerial plant tissues (Afzal et al., 2014; Khan et al., 2013; Weyens et al., 2009).

A major limitation of CWs regardless of their capability to efficiently remove a variety of pollutants and toxic compounds from wastewater is the possible inhibition of plant development, microbial proliferation, and pollutant degradation (Khandare et al., 2013b; Saleem et al., 2018; Shehzadi et al., 2014). To overcome these constraints, augmentation of CWs with pollutant-degrading and plant growth-promoting bacteria has been proposed (Shehzadi et al., 2014). These augmented bacteria are chosen because of the presence of specific genes and metabolic activities in them that enable them to degrade pollutants more efficiently than the indigenous microorganisms (Afzal et al., 2011; Ijaz et al., 2015; Rehman et al., 2018). Moreover, plant growth-promoting traits of the augmented bacteria help reduce stress due to contaminants and increase plant growth and development (Yousaf et al., 2011; Fatima et al., 2015; Shehzadi et al., 2016). Consequently, enhanced plant growth and microbial population in different components of CWs improve remediation of industrial wastewater (Arslan et al., 2017).

Halophytic plants, particularly halotolerant helophytes, contribute significantly to the ecosystem' remediation through a variety of physiological and/or biochemical processes (Syranidou et al., 2017; Wiessner et al., 2006). This includes (1) altering the microhabitat to make it appropriate for contaminant removal, (2) transferring atmospheric oxygen to plant rhizosphere that help bacterial communities to establish biofilms, (3) filtering/trapping the suspended particles for subsequent degradation, and (4) allowing endophytic bacteria to degrade contaminants taken up by the plant (Syranidou et al., 2017). *Leptochloa fusca* L. also known as a Kallar grass, is a halotolerant grass, whose lab-scale application has revealed its promise as a phytoremediative grass in bacterially-assisted CW systems (Ashraf et al., 2018). Its extensive root system allows it to grow and thrive in harsh environmental conditions, including waterlogged soils. It can be vegetated via its seeds, root cuttings, root stumps, and rhizomes; the shoot length can increase up to 1.5 m. The species grow well in summers, especially in the rainy seasons.

According to a recent dialogue published by the Proceedings of the National Academy of Sciences, commercialization of phytoremediation is lagging behind conventional technologies since it lacks reports of pilot-scale applications, and thus, loses industry's confidence to employ it as a reliable means of effluent treatment (Beans, 2017). To address this issue, we have attempted to establish a pilot-scale horizontal flow constructed wetland (CW) system that employs plant-endophyte partnership for the on-site remediation of dye-rich textile wastewater. The system was vegetated with *L. fusca* and inoculated with a consortium of endophytic bacteria were tested for effectiveness in water quality improvement. We also compared the nutrient removal performance of HFCWs with a vertical flow constructed system (VFCWs) that were run in parallel (Hussain et al., 2018). Persistence and activity of the bacteria were monitored in the wastewater as well as rhizo- and endosphere of the plant. The toxicity of wastewater was also determined by fish toxicity assay.

2. Material and methods

2.1. Textile effluent sampling and analysis

Wastewater effluent was collected from the equalization pond of Interloop Limited Khurrianwala, Faisalabad, Pakistan (coordinates: 31° 29′ N, 73° 17′ E). The company is one of the world's largest hosiery manufacturers, which is equipped with in-house spinning, yarn dyeing, knitting, and finishing facilities. The effluent was characterized for various physiochemical parameters, such as color, pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), nitrogen (N), phosphorous (P), total suspended solids (TSS), total dissolved solids (TDS), total settleable solids, (TSeS), and heavy metals (HMs) by using standard methods (Eaton et al., 2005). Moreover, toxicity of the wastewater samples was determined by fish toxicity assay as described previously (Afzal et al., 2008; Ijaz et al., 2015). Briefly, 5 healthy fish (*Labeo rohita*) were exposed to the treated and untreated textile effluent and the mortality rate was determined at different time intervals.

2.2. Selection of endophytic bacteria

Several endophytic bacterial strains that were previously isolated by Shehzadi et al. (2016) from the endosphere of *Typha domingensis* and *Pistia stratiotes* were tested for their potential to degrade the wastewater from Interloop. Briefly, their pure cultures were grown separately in Luria Bertani (LB) broth at 30 °C for 24 h. The bacterial pellet was harvested by centrifugation and then re-suspended in sterile 0.9% NaCl solution. The optical density was adjusted (10^7 cells ml^{-1}) for each strain using the turbidimetric method (Sutton, 2011). Subsequently, 10 ml of the suspension was inoculated in filter-sterilized wastewater (200 ml), and incubated at 30 °C for 10 days. Color removal and COD, BOD reductions were recorded every 2 days to study the pollutant degradation potential of each strain.

The *in vitro* performance of three bacterial strains namely *Microbacterium arborescens* TYSI04, *Pantoea* sp. TYRI15, and *Bacillus endophyticus* PISI25 was outstanding and therefore were selected for the inoculum preparation (Supplementary Tables 1 and 2). A successful potential in nutrients removal mainly N and P was also observed (data not shown). The inoculum was prepared as described previously: Each bacterial strain was cultured separately at 37 °C in LB broth, cells were harvested, re-suspended, and optical density was adjusted (CFU: 10^7 cells ml^{-1}) prior to mixing for the bacterial consortium. One liter of this consortium was then used as an inoculum in the CWs according to the experimental design.

2.3. Construction of HFCWs

The HFCWs were established in the vicinity of the wastewater treatment plant of Interloop Limited Khurrianwala, Faisalabad. Plastic containers (dimensions: 4 × 4 × 4 ft) with a water holding capacity of 1000 L were used to develop the HFCWs. Plastic sheets with holes were put in the tanks vertically to separate the coarse gravel (50 cm), fine gravel (30 cm), and sand (15 cm) (Fig. 1). The textile effluent level was maintained at 5–15 cm below the surface to ensure subsurface flow whereas a wide pipe was used to evenly distribute the flow.

A total of 24 HFCWs were prepared to set up 4 treatments with each treatment having 6 replicates. The treatments were: HFCWs containing tap water and vegetation (T1: control); HFCWs containing textile effluent without vegetation (T2: control); HFCWs containing textile effluent and vegetation (T3); HFCWs containing textile effluent, vegetation, and bacterial inoculation (T4). Cuttings of L. *fusca* were obtained from Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. One hundred cuttings were vegetated in each HFCW that were