Science of the Total Environment 645 (2018) 966–973

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Treatment of the textile industry effluent in a pilot-scale vertical flow constructed wetland system augmented with bacterial endophytes

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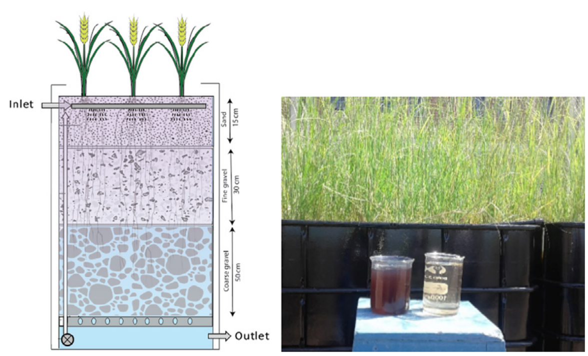
HIGHLIGHTS

- A pilot-scale endophyte-assisted VFCWs were engineered to treat real textile effluent.
- Endophytes augmentation increased removal of organic and inorganic pollutants.
- Inoculated bacteria displayed persistence in the root and shoot of *Brachiaria mutica*.
- This study recommends field-scale application of endophyte-assisted phytoremediation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 May 2018
Received in revised form 1 July 2018
Accepted 13 July 2018
Available online xxxx

Keywords:
Endophytes
Phytoremediation
Pilot-scale
Plant-bacteria partnership
Textile industry
Wastewater
VFCWs

ABSTRACT

A pilot-scale vertical flow constructed wetland (VFCWs) system was designed, implemented and operated for one year for the treatment of dye-rich real textile effluent. *Brachiaria mutica* was vegetated to develop VFCWs in which five different textile effluent degrading endophytic bacteria were inoculated. These bacteria were screened based on their dye degrading and plant growth promoting capabilities. The system's performance was evaluated by monitoring physicochemical parameters, nutrients removal, heavy metals reduction, detoxification potential, and persistence of endophytic bacteria in the plant rhizo- and endosphere. Although VFCWs were able to remove a majority of the pollutants from the wastewater, bacterial augmentation further enhanced the remediation efficiency. The system promoted an increase in dissolved oxygen up to 188% and, concomitantly, a substantial decrease in the chemical oxygen demand (81%), biochemical oxygen demand (72%), total dissolved solids (32%), color (74%), nitrogen (84%), phosphorous (79%), and heavy metals [Cr(97%), Fe(89%), Ni(88%), Cd (72%)] was recorded. Wastewater treated with VFCWs augmented with bacteria was found to be non-toxic and inoculated bacteria showed persistence in the root and shoot interior of *B. mutica*. Conclusively, VFCWs proved to be an effective methodology for treatment of textile effluent whereas its smaller size with high efficiency is an advantage for field-scale applications.

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https://doi.org/10.1016/j.scitotenv.2018.07.163
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1. Introduction

Textile industry contributes a major share in the economic development of developing countries. However, wastewater produces during fabric processing is one of the foulest polluters of water and soil (Kadam et al., 2018; Kim et al., 2006). It contains a variety of chemicals and auxiliaries such as dyestuff, heavy metals, pigments, detergents, salts, sulfur compounds, and oil and grease, etc. These chemicals are of serious health concerns due to their toxic, mutagenic, or carcinogenic nature (Bafana et al., 2009; Khataee et al., 2010). Additionally, they can lead to the degradation of environmental systems' in which they are discharged; for instance, surface water receiving dye-rich textile effluent suffers from light penetration and develops poor oxygen conditions (Khan and Malik, 2014). Similarly, presence of color in the textile effluent decreases aesthetic value of the receiving system (Delee et al., 1998).

Constructed wetlands (CWs) are eco-friendly and low-cost alternatives for the treatment of industrial wastewater. In a horizontal flow CW system, influent is fed from one side of the wetland whereas treated water is collected from the opposite side. This keeps the entire medium saturated with water and hence there are limited opportunities for contact between air and water (Kadlec and Wallace, 2008). Resultantly, exposure to oxygenated conditions in the rhizosphere for bacterial community is also limited (Schröder et al., 2008; Shehzadi et al., 2014). To enhance the degradation potential in such systems, large space is required for the on-site treatment of industrial wastewater. By contrast, vertical flow constructed wetlands (VFCWs) require less space because the system works more aerobically. Influent is loaded from the top and the aeration takes place during loading period. The contact between water and air is increased and aerobic zones are developed in the substrata. The plant roots favor microbial proliferation ultimately leading to successful aerobic degradation of wide range of organic compounds.

Textile effluent is rich in toxic compounds that can inhibit plant growth and microbial proliferation, consequently affecting the remediation efficiency (Kabra et al., 2013; Khandare et al., 2013; Shehzadi et al., 2014). To reverse the effect of these toxic compounds, the combined application of plant and contaminant degrading bacteria is proposed recently (Arslan et al., 2017; Khandare et al., 2013). Plants provide residency and nutrients to the rhizo- and endophytic bacteria whereas bacteria help their host to gain more biomass by reducing contaminant stress and performing plant growth-promoting services (Khan et al., 2013). The endophytic bacteria, however, have received more attention in the context of phytoremediation as they develop an intimate relationship with the host as compared to the rhizobacteria (Compant et al., 2010; Germaine et al., 2009; Mitter et al., 2013; Ryan et al., 2008). When equipped with appropriate pollutant degradation pathways, their application can reduce phytotoxicity as well as evapotranspiration of volatile organic compounds (Andria et al., 2009; Shehzadi et al., 2014). Ultimately, a major fraction of the toxic compounds are decontaminated in a relatively short time period when compared with the individual partners alone (Arslan et al., 2017).

The commercialization of phytoremediation is lagging behind, as less information is available about treatment success at pilot/field-scale. The need of time is to get back the industry's confidence by developing cost-effective, efficient and environmental friendly solutions for the on-site treatment. In this study, we developed and evaluated the performance of a pilot-scale endophyte assisted vertical flow constructed wetlands (VFCWs) in the vicinity of Interloop limited, Faisalabad, Pakistan (largest hosiery manufacturers), for the remediation of textile effluent. Previously, VFCWs are proposed for wastewater with high pollution load and poor dissolved oxygen (Villar et al., 2012). The system is applicable to the developing countries where capital and operational cost are very important (Mbuligwe, 2005a; Mbuligwe, 2005b; Prochaska et al., 2007). We hypothesized that VFCWs allows better colonization of bacterial endophytes in different compartments of the wetland leading to efficient degradation of textile effluent and reduction in toxicity.

2. Materials and methods

2.1. Wastewater characterization

Wastewater generated from the Interloop Limited Khurrianwala, Faisalabad, Pakistan is heavily polluted in terms of both organic and inorganic contaminants. The facility is equipped with in-house spinning, yarn dyeing, knitting and finishing processes; and its effluent contains dyestuff, heavy metals, and wide range of organic contaminants (Shehzadi et al., 2014). We collected wastewater from the equalization tank and then subjected to characterization by assessing various physiochemical and pollution parameters such as color, dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), total settleable solids (TSeS), total suspended solids (TSS), phenol, nitrogen, phosphorus, and heavy metals (Fe, Ni, Cr, Cd, and As) by using standard methods (Table 1). Toxicity was also determined by using fish toxicity assay as described previously (Afzal et al., 2008).

2.2. Selection of endophytic bacteria and inoculum preparation

In this study, twenty endophytic bacteria were tested for their potential to degrade textile contaminants. The strains were previously isolated from the plant interior (roots and shoots) of *Typha domingensis* and *Pistia stratiotes* by Shehzadi et al. (2016). Their pure cultures were grown separately in Luria Bertani (LB) broth in a shaker at 30 °C for 24 h. Subsequently, bacterial cells were harvested by centrifugation (10,000×g) and then re-suspended in sterile 0.9% sodium chloride solution. The amount (cells per ml) of each bacterial strain in the suspension was adjusted according to the turbidimetric method (Sutton, 2011). Twenty ml suspension (10^7 CFU ml^{-1}) of each bacterial strain was inoculated in filter-sterilized Interloop wastewater (200 ml).

All of the bacterial strains exhibited Interloop effluent degrading activities (Table 1). A significant decrease in color, COD, and BOD was observed by bacterial inoculation after 72 h of incubation. *Pantoea* sp. strain TYRI15 removed highest amount of color, COD, and BOD (i.e., 35, 42 and 44% respectively) as compared to the control treatment. Nevertheless, 4 other bacterial strains also displayed effluent degradation capabilities, and these were: *Microbacterium arborescens* TYSI04; *Bacillus endophyticus* PISI25; *Bacillus pumilus* PIRI30 and *Bacillus* sp. TYSI17 (Table 2). These 5 strains were cultivated separately in LB broth at 37 °C to prepare the inoculum. Their cells were harvested by centrifugation and then re-suspended in sterile 0.9% sodium chloride solution. The numbers of bacterial cells were adjusted and inoculum

Table 1
APHA methods used to assess the wastewater quality parameters.

Parameter	APHA method
Color	Method 2120
Dissolved oxygen (DO)	Method 4500-O C.
Chemical oxygen demand (COD)	Method 5220 D
Biochemical oxygen demand (BOD)	Method 5210 D
Total dissolved solids (TDS)	Method 2540C
Total suspended solids (TSS)	Method 2540 D
Total settleable solids (TSeS)	Method 2540 F
Phenol	Method 5530 D
Fe	Method 3500-Fe B
Ni	Method 3500-Ni B
Cr	Method 3500-CR B
Cd	Method 3500-CD
As	Method 3500-AS
Nitrogen (N)	Method 4500-N
Phosphorus (P)	Method 4500-P

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