Ecotoxicology and Environmental Safety 161 (2018) 70–77

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Asparagus densiflorus in a vertical subsurface flow phytoreactor for treatment of real textile effluent: A lab to land approach for *in situ* soil remediation

Anuprita D. Watharkar[a,b,1], Suhas K. Kadam[a,1], Rahul V. Khandare[b,*], Parag D. Kolekar[c], Byong-Hun Jeon[d], Jyoti P. Jadhav[a,c], Sanjay P. Govindwar[a,d,**]

[a] Department of Biochemistry, Shivaji University, Kolhapur, India
[b] Amity Institute of Biotechnology, Amity University, Mumbai, India
[c] Department of Biotechnology, Shivaji University, Kolhapur, India
[d] Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, South Korea

ARTICLE INFO

Keywords:
Phytoremediation
A. densiflorus
Rubin GFL
Textile effluent
Vertical subsurface flow phytoreactor
Toxicity

ABSTRACT

This study explores the potential of *Asparagus densiflorus* to treat disperse Rubin GFL (RGFL) dye and a real textile effluent in constructed vertical subsurface flow (VSbF) phytoreactor; its field cultivation for soil remediation offers a real green and economic way of environmental management. *A. densiflorus* decolorized RGFL (40 gm L^{-1}) up to 91% within 48 h. VSbF phytoreactor successfully reduced American dye manufacture institute (ADMI), BOD, COD, Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) of real textile effluent by 65%, 61%, 66%, 48% and 66%, respectively within 6 d. Oxidoreductive enzymes such as laccase (138%), lignin peroxidase (129%), riboflavin reductase (111%) were significantly expressed during RGFL degradation in *A. densiflorus* roots, while effluent transformation caused noteworthy induction of enzymes like, tyrosinase (205%), laccase (178%), veratryl oxidase (52%). Based on enzyme activities, UV–vis spectroscopy, FTIR and GC-MS results; RGFL was proposed to be transformed to 4-amino-3- methylphenyl (hydroxy) oxoammonium and N, N-diethyl aniline. Anatomical study of the advanced root tissue of *A. densiflorus* exhibited the progressive dye accumulation and removal during phytoremediation. HepG2 cell line and phytotoxicity study demonstrated reduced toxicity of biotransformed RGFL and treated effluent by *A. densiflorus,* respectively. On field remediation study revealed a noteworthy removal (67%) from polluted soil within 30 d.

1. Introduction

Textile industries consumes large amount of water as a primary medium in various processes like desizing, scouring, bleaching, dying, printing and finishing. Nearly 15–20% of the synthetic dyes and other chemicals used during such dyeing practices are released as a process discharge and lead to secondary disposal problems. These intensely colored liquid waste pollutes our valuable water bodies and agricultural lands, and affects all biological forms of the ecosystems even at very low concentrations (Spadaro et al., 1992; Kabra et al., 2011). Textile wastewater constituting large variety of synthetic dyes, supplementary binding chemicals, resins, wax, acid, alkali, heavy metal salts and residual chlorine is posing as a potent challenge to the environment. Residual chlorine released during bleaching serve as a strong oxidizing agent. It combines with other impurities to form toxic substances and ultimately lowers the dissolved oxygen of receiving water body. Textile effluents are loaded with recalcitrant xenobiotics compounds and therefore resistant against conventional degradation methods due to their superior color and light fastness (Stolz, 2001). Further, such wastewaters are not only toxic to aquatic life forms but also harmful to humans who use the contaminated river waters (Wang et al., 2009). Therefore, new cost effective, reliable and environmental technologies for abatement of water pollution needs to be developed and practically adopted.

Microbial remediation strategies are competent but known to be inapplicable due to high cost, technical constraint and secondary sludge formation. Thus, it is necessary to employ alternate economic and viable methods for the ecofriendly treatment of complex textile effluent. Currently, phytoremediation is propagating as a capable green technology for the reclamation of polluted sites and wastewater over conventional physicochemical and photochemical degradation methods. This is an autotrophic as well as aesthetically gratifying tool for

* Corresponding author.
** Corresponding author at: Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, South Korea.
E-mail addresses: rvkhandare@mum.amity.edu (R.V. Khandare), spgovindwar@hanyang.ac.kr (S.P. Govindwar).
[1] Authors have equal contribution.

https://doi.org/10.1016/j.ecoenv.2018.05.078
Received 1 February 2018; Received in revised form 18 May 2018; Accepted 28 May 2018

biodegradation of textile effluent with low cost and significant effectiveness (Khandare and Govindwar, 2015). Phytoremediation with pilot or large scale hydroponic systems as well as phytoreactors along with other more conventional remedial methods can be used as a final/polishing step of treatment (Schwitzguébel et al., 2002). This includes *in situ* or *ex situ* cultivation of plants within defined range of contamination for the requisite period of stabilization and growth. Additionally, rhizospheric bioremediation of xenobiotic compounds such as textile dyes from the polluted water and soil can also be accomplished.

Several reports on a variety of constructed phytoreactors to treat real textile wastewater are now available. To mention a few, biodegradation of Navy Blue HE2R, a real textile effluent and a simulated dye mixture by *Portulaca grandiflora* was reported (Khandare et al., 2013, 2011). A bacterial assisted static hydroponic phytoreactor with bamboo grass plantation was applied for the treatment of textile wastewater (Watharkar et al., 2015). Macrophytes *Alternanthera philoxeroides* and *Ipomoea aquatica* were reported to degrade sulfonated dye Remazol Red and Brown 5 R, respectively. Further, they were utilized for decolorization of textile effluent at pilot scale rhizofiltration units separately as well as combinatorial hybrid reactors and also in *in situ* constructed lagoons (Rane et al., 2016, 2015). Large scale textile effluent treatment by *Salvinia molesta* in shallow lagoons were reported to reduce the values of COD (76%), BOD (82%) and ADMI (81%) (Chandanshive et al., 2016). *Typha angustifolia, Paspalum scrobiculatum* and their co-plantation have also been employed for a field trials and remediation experiments with built drenches and exhibited an efficacious removal of azo dyes from effluent (Chandanshive et al., 2017). Co-planted floating phyto-beds of *Fimbristylis dichotoma* and *Ammannia baccifera* were also found to achieve notable reductions in ADMI color value (79%), COD, (72%), BOD (77%), TDS (66%) and TSS (56%) of textile effluent (Kadam et al., 2018).

This work involves the employment of *A. densiflorus* to degrade and detoxify disperse RGFL dye as well as *ex situ* real textile wastewater treatment using constructed vertical subsurface flow (VSbF) phytoreactor. *In situ* plantation of *A. densiflorus* along with high rate transpiration system (HRTS) was carried out to perform soil remediation studies at Maharashtra industrial development corporation (MIDC) of Kagal, India. *A. densiflorus* is an evergreen, perennial ornamental plant. It gives a cushion like foliar appearance and has long, arching stems with dense dark green, needle-like leaves. It possesses immense and tuberous structured advanced root system which makes it a suitable applicant for phytoremediation. It is self-vegetative and fast growing. It can simply be cultured by sowing seeds or separate clump of tuber root as mature specimen. The plant has economic value as the foliage can also be integrated as filler with flowers in decorative arrangements. Yang et al. (2009) have shown that *A. densiflorus* possesses phytovolatization potential and can take away benzene, octane, α-pinene, toluene, trichloroethylene (0–13.28 $\mu g/m^3/m^2/h$) from air. Removal efficiency of plants by volatilization is mainly based on leaf area of a selected plant. It also varies with chemical properties of the VOC such as molecular weight, vapour pressure, polarity, solubility etc. Similarly, the fate of VOC is depending upon physical properties such as adsorption, absorption, penetration, accumulation, metabolism and volatilization. Lipophilic nature of these VOCs probably makes them bioavailable and expedite penetration to cuticular surface of *A. densiflorus*. Hence, this plant with leaf area 337 ± 9 cm^2/plant might have exhibiting superior removal efficiency of these volatile compounds. This study reveals its dye and effluent phytoremediation potential.

2. Materials and methodology

2.1. Dyes and chemicals

Textile dyes namely Red HE3B, Yellow Brown REL, Direct Blue, Brown 3, Golden Yellow HER, Green HE4B, Scarlet RR and RGFL used for screening purpose were purchased from Mahesh dye processing company, Ichalkaranji, India. The dyes purity was approximately 85%. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Sigma-Aldrich (St Louis, MO, 140 USA). N-propanol, catechol, tartaric acid, ascorbic acid, disodium salt, azo dye methyl red, riboflavin, nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) were procured from Sisco Research Laboratories, Mumbai, India. Chemicals used in this study were of highest purity and analytical grade.

2.2. Collection of A. densiflorus and decolorization experiments

Several ornamental herbs were subjected for color removal trials with a few arbitrarily selected dyes. *A. densiflorus* from Asparagaceae family was selected as a potential phytoremediator based on its performance of dyes decolorization among all other plants. It is a non-edible, fast growing, scrambling, and slightly woody plant with advanced tuberous root system and trailing branches up to 1 m long. Its tiny foliage gives a fluffy and bright green appearance and can be used in flower bouquet combination with plants having medium or very large leaves to achieve textural contrast. *A. densiflorus* is available in large amount throughout the year and is not hazardous to an environment. Nursery grown plants were bought from Sajeev Nursery, Kolhapur, India.

2.3. Decolorization study

2.3.1. Decolorization of RGFL by A. densiflorus

Initially screening was performed with the nursery grown *A. densiflorus* plants using various dyes such as Red HE3B, Yellow Brown REL, Green HE4B, Direct Blue, Brown 3, Golden Yellow HER, Scarlet RR and RGFL. Root soil of *A. densiflorus* was washed off and plant roots were subjected to 0.1% $HgCl_2$ (w/v) and distilled water wash for 2 min each. Primary color removal experiments were carried out using single plant having a total biomass of 70 ± 4 g in 500 mL beaker having 200 mL of 20 mg L^{-1} of dye solution in distilled water. Absorbance of all dyes solution were observed individually after an interval of 6 h by removing 1 mL of each solution. These solutions were centrifuged at 4561*g* for 15 min; supernatants were subjected to their corresponding wavelengths of maximum absorption and decolorization percentages were estimated (Khandare et al., 2012). RGFL was selected as the model dye considering disperse and complex nature, to conduct further experiments. Abiotic control i.e. plain dye solution as well as the biotic control i.e. plants roots immersed in distilled water were also kept for better comparative results. Absorbances of the RGFL solution at various concentrations like 20, 40, 60, 80 and 100 mg L^{-1} were measured after every 12 h by removing 1 mL of solution at 480 nm up to 48 h and maximum percent decolorization was calculated.

2.4. Studies of enzyme assays and anatomy of root during color removal

Advanced tuberous roots of the *A. densiflorus* exposed to distilled water (control A), tap water (control B), RGFL (test A) and textile effluent (test B) were used for making crude enzymatic solutions. Separated and crushed root tissues in 50 mM potassium phosphate buffer (pH 7.4) were homogenized and centrifuged at 8481*g* for 15 min at 4 °C. Thus, the cell-free cocktail obtained after collecting supernatant was used as a source of intracellular enzymes. Lowry's method was utilized to estimate the protein content of all samples (Lowry et al., 1951).

All oxidoreductive enzymes in control and test samples were assayed at 30 °C along with their reference blanks that contained all reactants except the enzyme. For estimating specific activities of these enzymes, difference between corresponding absorbances at initial (0 s) to final (60 s) value at their respective λ_{max} and total protein content per milliliter were considered. Lignin peroxidase, laccase, veratryl