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Phytoremediation of textile effluent pretreated with ultrasound and bacteria

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ABSTRACT

This study presents the phytoremediation of textile effluent pretreated with ultrasound irradiation and microbial treatment. The isolates such as *Ochrobactrum* sp., *Pseudomonas aeruginosa* and *Providencia vermicola* were used for the bacterial degradation and phytoremediation was done using *Bouteloua dactyloides*. The effect of sonication time on the performance of phytoremediation was evaluated. *Ochrobactrum* sp. showed better performance compared to other species and better results were observed with the effluent sonicated for a longer period. The pretreatment of textile effluent with *Ochrobactrum* sp. and sonication improved the phytoremediation and showed about 95% COD reduction at the end of 24 h. The influence of oxidoreductive enzymes such as laccase, NADH–DCIP reductase and azoreductase during bacterial degradation was studied and the activity of catalase and peroxidase was identified during phytoremediation. The UV–visible and high performance liquid chromatography analysis confirmed the degradation of dye molecules. The possible metabolic pathway was identified through liquid chromatography-mass spectroscopy analysis. The X-ray diffraction (XRD) analysis showed the reduction in the heavy metals content of the treated effluent and the phytotoxicity analysis confirmed the less toxic nature of the treated effluent.

1. Introduction

The textile industries require significant quantity of water and chemicals for the wet processing of textiles. The chemical reagents used are very diverse in composition, which are ranging from inorganic compounds to polymers and organic products [1]. The presence of even very less amount of dyes in the effluent is highly visible and undesirable [2]. The conventional treatment methods such as coagulation–flocculation and adsorption are not very efficient. These methods produce a large amount of sludge, which is highly undesirable. The secondary pollutants generation needs to be avoided for the sustainable development. Therefore, the development of new technologies for treatment of textile effluent is inevitable. In the recent past, ultrasound based methods have gained much importance [3,4].

Sonolysis has been successfully used to treat wide range of complex organics and the hydroxyl radicals produced during sonolysis are responsible for the degradation of such compounds. However, the high operating cost associated with ultrasound based methods limits its commercialization [5]. Hence, the integrated methods, which combine ultrasound/Fenton processes with biological treatment, have been developed and such methods were reported to be more effective [6].

Phytoremediation is the use of plants for the degradation of both soil and water contaminants. In particular, rhizoremediation was found to be efficient for the treatment of several organic contaminants prevailing in the industrial environment. Generally, the plants have a unique defense mechanism to survive during environmental changes by the formation of Reactive Oxygen Intermediates (ROIs). On the other hand, plants and the microbes have the capacity to survive in the toxic environment by adaptive response process [7]. Phytoremediation can be used to treat heavy metals, pesticides, chlorinated solvents, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and radionucleotides [8]. Plants such as *Typha angustifolia, Blumea malcolmii, Tagetes patula, Aster amellus, Glandularia pulchella* and *Sesuvium portulacastrum* were successfully used to treat textile dyes such as Malachite green, Reactive Red 2, Direct Red 5B and Green HE4B [9–12]. The plant used in this study, *B. dactyloides* (Buffalo grass), is a perennial grass and possesses a fibrous root system. This grass has the ability to reduce the total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) present in the soil by rhizodegradation/bioaccumulation [13].

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However, phytoremediation is a slow process and the rate can be increased by pretreating the effluent with suitable method. The treatment methods, which combine ultrasound or Fenton process with microbial treatment, have been reported in the literature [14,15]. The scope of the present study was to study the phytoremediation of textile effluent pretreated with sonication and bacteria. This study also explores the role of different enzymes responsible for the degradation of effluent during bacterial degradation and phytoremediation.

2. Materials and methods

2.1. Sonication

A bath-type sonicator (36 kHz, 3.5L100H, PCI Analytics, India) with the operating frequency of 30 kHz was used in the present study. The sonicator tank was made of stainless steel, and the bottom of the tank was fitted with an ultrasonic transducer. The timer and temperature indicator were provided with the sonicator system. The textile effluent was placed in the sonicator and ultrasound irradiation was done for the desired period.

2.2. Microorganism

Bacterial strains used in the present study were isolated from soil samples collected from textile effluent disposal sites, located in the southern part of India. The strains were isolated by standard serial dilution method. Serial dilution method is used to dilute the concentrated complex bacterial culture obtained from natural sources such as soil and water. The samples were diluted with distilled water from 10^{-1} to 10^{-6} dilution. From the dilution factor of 10^{-4} and 10^{-5}, 0.1 ml samples were withdrawn and incubated in nutrient agar plates for 24 h. This range contains a nominal amount of microbes and hence, it was chosen. The individual colonies grown in the Petri dishes were further isolated based on the morphological characteristics and sub-cultured again to obtain pure isolates. The bacterial strains were selected based on their ability to decolorize the dyes present in the nutrient agar plates. The bacterium was identified, based on morphological, biochemical characteristics, and by 16S rRNA sequencing. The pure culture obtained was maintained in a nutrient agar slant at 4 °C. The culture was retrieved by sub-culturing in a 250 ml Erlenmeyer flask containing 100 ml nutrient broth for 24 h under shaking conditions.

2.3. Biodegradation study

A number of common grass varieties were screened for the treatment of diluted effluent. Among the grass varieties tested, *B. dactyloides* was found to grow well and adapt to the effluent, and hence, this was chosen for the further study. *B. dactyloides* belongs to the Poaceae family and is a fast growing lawn grass. This is available throughout the year and causes no environmental hazard. The wild plants were collected from the Anna University campus, Chennai, India. *B. dactyloides* of 0.380 g weight and 14 cm height was washed with running tap water, to remove the soil debris and then treated with 0.1% $HgCl_2$ (w/v) for 2 min to remove any contaminants. Finally, the roots were washed with distilled water. The degradation experiments were performed in 250 ml beakers containing 100 ml effluent and ten uprooted plants. The performance was assessed based on COD reduction.

During the sequential treatment, the effluent was pretreated in the sonicator for the desired period. The application of ultrasound irradiation moderately increased the temperature of the effluent. However, the effluent was left undisturbed for 20 min at room temperature after sonication and then it was subjected to phytoremediation (24 h). In the bacterial assisted phytoremediation, the effluent was pretreated with the log phase *Ochrobactrum* sp., *Pseudomonas aeruginosa*, and *Providencia vermicola* for 12 h (without any filtration), and then subjected to phytoremediation (12 h). The degradation was expressed as % COD reduction and was calculated using Eq. (1).

$$\%\text{CODreduction} = \frac{\left(\text{COD}_{(\text{initial})} - \text{COD}_{(t)}\right)}{\text{COD}_{(\text{initial})}} \times 100$$

where $\text{COD}_{(\text{initial})}$ and $\text{COD}_{(t)}$ represent the initial COD and the COD at time '*t*' (h), respectively. Abiotic controls (without microorganism) were included during the experimental investigation.

2.4. Preparation of cell-free extract

To study the enzyme assay, the roots of *B. dactyloides* before and after treatment were excised from the plants, chopped finely, suspended in 50 mM potassium phosphate buffer (pH 7.4), which was grounded in a pestle and mortar, and finally centrifuged at 8480 × *g* for 20 min. The supernatant obtained was used as an enzyme source [16].

The enzyme study was performed for the most efficient microbe among the isolates studied and the cell free extract obtained was centrifuged at 10,000 × *g* for 20 min at 4 °C. The pellet obtained was suspended in 50 mM potassium phosphate buffer (pH 7.4). The contents were gently homogenized and sonicated (30 s, 40 Amplitude and 7 strokes) at 4 °C. Then, the sonicated cells were centrifuged at 10,000 × *g* for 15 min at 4 °C and the resulting extract was used as an enzyme source. A similar procedure was followed for the cells obtained after degradation [16].

2.4.1. Enzyme assay

The peroxidase and catalase activities have been reported for the plants exposed to textile effluent [17], and hence, in the current study, the same were analyzed. For analyzing the peroxidase activity, a mixture containing 0.7 ml of solution 1 (prepared using 810 mg phenol and 25 mg 4-aminoantipyrene in 50 ml of distilled water), 0.1 ml of plant extract and 0.8 ml of solution 2 (0.01% of hydrogen peroxide in 0.01% M 4-(2 hydroxyethyl)-1-piperzine ethane sulfonic acid(HEPES), and buffer (pH 7.1)) was prepared. The absorbance was measured for 1 min at 510 nm. For analyzing the catalase activity, the assay mixture consisting of 0.1 ml of plant extract, 0.15% hydrogen peroxide buffered in potassium phosphate buffer (pH 7.6), and 0.9 ml of potassium phosphate buffer (0.05 M, pH 7.6) was prepared. The enzyme activity was measured at 240 nm for 1 min [17]. The activity of oxido-reductive enzymes before and after treatment was analyzed for the bacteria. Laccase, NADH–DCIP reductase and azoreductase activity were assayed spectrophotometrically at room temperature. The laccase activity was measured by observing the change in absorbance of a reaction mixture containing 10 mM Guaiacol in 100 mM of acetate buffer with 0.1 ml enzyme at 470 nm, and the enzyme activity was expressed in U/ml [18]. NADH dichlorophenol–indophenol (NADH–DCIP) reductase activity was determined by following the procedure reported in the literature [19]. The mixture containing 50 mM DCIP, and 50 mM NADH in a 50 mM potassium phosphate buffer (pH 7.4) was added with 0.1 ml enzyme. The DCIP reduction was monitored at 620 nm and calculated using the extinction coefficient of 19 mM/cm. For the azoreductase assay, the reaction mixture containing Methyl red (2 mM), 50 mM NADH in phosphate buffer (pH 7.0) and 0.1 ml enzyme was prepared [19]. One unit of enzyme activity was defined as the change in absorbance per ml of enzyme. All enzyme assays were carried out in triplicate, and the average values were reported. The protein content of the samples were determined by Lowry's method [20].