Ecotoxicology and Environmental Safety 114 (2015) 23–30

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Improved biodegradation of textile dye effluent by coculture

S.R. Vijayalakshmidevi, Karuppan Muthukumar *

Department of Chemical Engineering, Alagappa College of Technology Campus, Anna University, Chennai 600025, India

ARTICLE INFO

Article history:
Received 29 May 2014
Received in revised form
23 September 2014
Accepted 26 September 2014
Available online 14 January 2015

Keywords:
Textile effluent
Coculture
Biodegradation
Toxicity

ABSTRACT

The present study demonstrates the de-colorization and degradation of textile effluent by coculture consisting of three bacterial species isolated from textile effluent contaminated environment with an aim to reduce the treatment time. The isolates were identified as *Ochrobactrum* sp., *Pseudomonas aeruginosa* and *Providencia vermicola* by 16S rRNA analysis. Their secondary structure was predicted and GC content of the sequence was found to be 54.39, 52.10, and 52.53%. The co-culture showed a prominent increase in the degradation activity due to the action of oxidoreductase enzymatic mechanism of laccase, NADH–DCIP reductase and azoreductase activity. The biodegradability index of 0.75 was achieved with 95% chemical oxygen demand (COD) reduction in 16 h and 78 and 85% reduction in total organic carbon (TOC) and total solids was observed. Bioaccumulation of metals was identified by X-ray diffraction (XRD) analysis. The effective decolorization was confirmed from the results of UV–vis spectroscopy, high performance liquid chromatography and Fourier transformed infrared spectrometer analyzes. The possible degradation pathway was obtained from the analysis of liquid chromatography–mass spectroscopy analysis and the metabolites such as 2-amino naphthalene and N-phenyl-1.3,5 triazine were observed. The toxic nature of the effluent was analyzed using phyto-toxicity, cell-death assay and geno-toxicity tests.

1. Introduction

The textile industry is one of the largest water consuming industries and releases large amount of effluent (Hai et al., 2006). The textile effluent contains carcinogenic dyes, toxic heavy metals, phenolic compounds, softeners and other chemicals used in the dyeing process (Correia et al., 1994). During the dyeing process, about 50% of the dye remains with the spent dye bath effluent, in its hydrolyzed form, which loses its affinity towards the fabric that cannot be re-used in the dyeing process (Watanapokasin et al., 2008). This effluent has to be disposed safely since dyes are toxic, mutagenic and cause major health hazards. The treatment methods include chemical oxidation, coagulation–flocculation, membrane filtration, adsorption, photo-catalysis, biodegradation etc., Some of these processes are complex and expensive (Neill et al., 1999, Eichlerova et al., 2006). Biodegradation is a cost-effective process (Swamy and Ram, 1999) and a large variety of microorganisms such as bacteria, fungi, yeasts, actinomycetes and algae are capable of degrading dyes (Dafale et al., 2007). Microorganism can be used, either as a pure culture or as a consortium to degrade dye containing wastewater (Moosvi et al., 2005; Moosvi et al., 2007; Chen and Chang, 2007; Telke et al., 2008; Kalyani et al., 2009). Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Serratia* sp., *Yersinia* sp., *Erwinia* sp., *Alcaligenes* sp., *Sphingomonas* sp., *Enterobacter cancerogenus, Paenibacillus polymyxa* and *Micrococcus* sp. were successfully used for the degradation of different textile dyes (Moosvi et al., 2005, 2007; Khehra et al., 2005; Kumar et al., 2007; Dafale et al., 2007, Jiranuntipon et al., 2008; Tony et al., 2009; Gou et al., 2009; Jadhav et al., 2010). Several studies report that synergistic metabolic activities of mixed microbial consortium could completely mineralize dyes (Tony et al., 2009). For example, a bacterial consortium consisting of *Proteus vulgaris* and *Micrococcus glutamicus* was found to degrade dye containing effluent with 60% chemical oxygen demand (COD) reduction in 96 h (Saratale et al. 2010).

The reviews on biodegradation stated to require more incubation time to mineralize the dyes. Therefore, the present study focuses on the incubation time reduction and this is achieved using novel coculture consisting of microorganisms isolated from textile effluent. The comparison of the axenic strain and the coculture degradation efficiency was done. The toxicity analysis of the treated effluent evaluated by cell death assay,genotoxicity test andphytotoxicity study.

2. Materials and methods

2.1. Microorganism

The microorganisms isolated from the textile effluent contaminated environment were identified based on morphological

* Corresponding author. Fax: +91 44 22352642.
E-mail address: muthukumar@annauniv.edu (K. Muthukumar).

http://dx.doi.org/10.1016/j.ecoenv.2014.09.039
0147-6513/

and physiological characteristics and by 16S rRNA gene sequencing. The sequence similarity searches of partial 16S rRNA nucleotide sequences initially done using public nucleotide database using nBLAST. The corresponding sequences were submitted to Bankit, and the accession id retrieved for the isolates. The bacterial nucleotide sequences were aligned using multiple sequence alignment tool CLUXTAL X2 program (www.clustal.org) and sequence similarity among three novel isolates was viewed for an evolutionary relationship through phylogenetic tree using Mega 4.1 software (www.megasoftware.net). The bacterial strains isolated were maintained routinely on nutrient agar medium in the form of slants at 4 °C. Energy minimization was performed using the RNA secondary structure by using Kinefold program (Xayaphoummine et al., 2005). The G+C content of the sequence for each isolate was determined using GeneMark.hmm (Version 2.8) (Lukashin and Borodovsky, 1998).

2.2. Acclimatization

The acclimatization of microorganism to the textile effluent was done by incrementally increasing their exposure to the effluent. Initially, the acclimatization process was performed with nutrient broth containing 10% effluent at 37 °C in 250 ml conical flask. However, the nutrient broth concentration was decreased gradually from 90% to 0% until the organism provided with only the effluent as the sole source of nutrient.

2.3. Biodegradation studies

Biodegradation was carried out with Ochrobactrum sp., *P. vermicola* and *P. aeruginosa* as axenic culture and mixed culture. A loopful of each microorganism was inoculated into 50 ml of nutrient broth and incubated for 6 h. Then coculture was developed by mixing each culture together in a nutrient broth and the same was incubated for 6 h. About 20 ml of inoculum was then added into 250 ml Erlenmeyer flask containing 100 ml textile effluent (undiluted) and the contents were incubated in an orbital shaker at 150 rpm, at 37 °C.

The degradation expressed as % COD reduction was calculated using Eq. (1):

$$\%\text{COD reduction} = \frac{(\text{COD}_{(initial)} - \text{COD}_{(t)})}{\text{COD}_{(initial)}} \times 100 \quad (1)$$

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

2.4. Wastewater characteristics

The textile dye effluent was collected from a textile dyeing unit located in the southern part of Tamil Nadu, India. The color of the effluent was purple red. The performance of the treatment was assessed based on biological oxygen demand (BOD) and chemical oxygen demand (COD) reduction. The characteristics of the effluent such as hardness, alkalinity, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), pH, total kjeldal nitrogen (TKN), total organic carbon (TOC), electrical conductivity (E.C), were analyzed by following the procedure prescribed by APHA. The metal ion content in the untreated and treated effluent was analyzed using atomic absorption spectrometer (Perkin-Elmer-Analyst400).

2.5. Growth kinetics

The specific growth rate of the coculture was determined using the following equation:

$$\ln \frac{x}{x_o} = \mu t \quad (2)$$

where x is the concentration of biomass (g/L) at time (t) and x_o is the initial concentration of biomass (g/L) at time $t=0$ and μ is the specific growth rate (h^{-1}). The growth yield (Y) can be expressed as

$$\frac{dx}{ds} = Y \quad (3)$$

Eq. (3) can also be written as

$$x - x_o = Y(S_o - S) \quad (4)$$

where S_o is the initial concentration of substrate expressed as COD($mg\ l^{-1}$), S is the final concentration of substrate expressed as COD($mg\ l^{-1}$), x is the concentration of biomass ($mg\ l^{-1}$) and x_o is the initial concentration of biomass ($mg\ l^{-1}$)

2.6. Preparation of cell free extract

The coculture and axenic strains were grown in their respective medium for 24 h at 37 °C and centrifuged at 10,000 g for 20 min. The biomass obtained was suspended in 50 mM potassium phosphate buffer (pH 7.4) and gently homogenized. Then the content was sonicated in a sonlyzer with an output of 60 amplitude and 12 strokes each of 30 s with one min interval at 4 °C. The extract obtained was used as enzyme source. The similar procedure was followed for the cells obtained after degradation (Parshetti et al., 2006).

2.6.1. Determination of enzyme activities

The activity of oxido-reductive enzymes was analyzed for the axenic strain and coculture. The laccase, NADH dichlorophenol-indophenol (NADH–DCIP) reductase and azoreductase activity were assayed spectrophotometrically at room temperature. The laccase activity was measured by observing the 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^{-1} (Jadhav et al., 2008). NADH–DCIP reductase activity was determined by following the procedure reported in the literature (Jadhav et al., 2010). The reaction mixture containing 50 mM DCIP and NADH in 50 mM potassium phosphate buffer (pH 7.4) was added with 0.1 ml enzyme. DCIP reduction was monitored at 620 nm and calculated using the extinction coefficient of 19 mM/cm. For azoreductase assay, the reaction mixture containing 2 mM methyl red, 50 mM NADH in phosphate buffer (pH 7.0) and 0.1 ml enzyme was used (Jadhav et al., 2010). One unit of enzyme activity was defined as the change in absorbance unit per ml of enzyme. All enzyme assays were carried out in triplicate and the average values were reported.

2.7. Analytical studies

2.7.1. UV–vis spectrophotometric analysis

About 5 ml of untreated and treated effluent was filtered through 0.22 μm filter and was subjected to UV–vis spectral analysis (Elico Ltd., India) in the wavelength range from 200 to 800 nm.

2.7.2. HPLC analysis

The untreated and treated effluent were analyzed in HPLC (Shimatzu, Japan) equipped with UV–vis detector using C18 column (symmetry 4.6 mm × 250 mm), diode array, a quaternary