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# Exploring bioremediation strategies to enhance the mineralization of textile industrial wastewater through sequential anaerobic-microaerophilic process



Kshama Balapure <sup>a</sup>, Kunal Jain <sup>b</sup>, Nikhil Bhatt <sup>a</sup>, Datta Madamwar <sup>b, \*</sup>

<sup>a</sup> Post Graduate Department of Microbiology, Biogas Research and Extension Centre, Gujarat Vidyapith, Sadra, 382 320, Gujarat, India <sup>b</sup> Environmental Genomics and Proteomics Lab, BRD School of Biosciences, Satellite Campus, Vadtal Road, Post Box No. 39, Sardar Patel University, Vallabh Vidyanagar, 388 120, Gujarat, India

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# ABSTRACT

The study exemplifies sequential anaerobic-microaerophilic bioremediation process for treatment of textile industrial wastewater having 10,000 mg l<sup>-1</sup> of COD and 3330 mg l<sup>-1</sup> of the BOD. The experimental results showed that, in an anaerobic phase, with cattle dung slurry as an initial feed, nearly 60% of COD and BOD was removed from textile wastewater at an optimum HRT of 2d and OLR of 5.0 kg COD m<sup>-3</sup>d<sup>-1</sup>. Further, COD and BOD removal efficiency of bacterial consortium BDN was enhanced upto 97% under microaerophilic phase, at HRT of 12 h. Moreover, optimum color removal (80%) was observed in anaerobic reactor. The combine treatment process removed 99% of color at combine HRT of 60 h. The activity of lignin peroxidase was higher as compared to other enzymes studied. The UV–vis, FTIR, <sup>1</sup>H NMR and GC–MS analyses of treated textile industrial wastewater revealed the degradation of dye compounds and formation of lower molecular weight intermediates. The toxicity of textile industrial wastewater decreased subsequently from anaerobic to microaerophilic treatment process.

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# 1. Introduction

The textile industry, apart from being an important contributor to the economy of the many countries, is also a major source of various liquid and solid wastes. These industrial wastes are considered as a harmful pollutant, which are released into the natural water resources or wastewater treatment systems (Rondon et al., 2015). It was estimated that more than 80,000 tons/year of dyes are consumed in textile dyeing processes, which requires 70–150 dm<sup>3</sup> of water and 40 g of reactive dyes per kg of cotton (Mendez-Martineza et al., 2012). The amount of water consumed and released also varies depending on the type of fabrics used. Therefore, the composition of the dye wastewater varies with the type of textile produced (Mustafa and Delia, 2004). The entry of these pollutants into water streams poses a severe ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect human beings through the food chain (Mohana

\* Corresponding author.

# et al., 2008).

Due to strict government legislation and regulation, textile wastewater remediation is a deeply studied topic worldwide. Moreover, several new and improved methods are also being developed continuously to remove one or more xenobiotic dyes from industrial textile wastewater. These methods include physical, chemical and advanced chemical oxidation treatment. However, considering the pitfalls of these techniques during practical implementation, the process itself needs further optimization in terms of quality, applicability and cost (Bhatt et al., 2005). Therefore, bioremediation using bacteria is gaining importance as it is cost effective, ecofriendly, and produce negligible sludge (Jain et al., 2012). Different taxonomic groups of bacteria have been reported for their ability to degrade azo dyes (Moosyi et al., 2007). Bacteria have sets of catabolic genes, capable of processing various metabolic pathways, which are integrated in such a manner that xenobiotic compounds (such as dyes) are converted to low molecular weight intermediates which can enter into central metabolic pathway leading to complete mineralization of those compounds including dyes.

Azoreduction by sequential anaerobic-aerobic process is most commonly used worldwide, due to its simplicity and low cost

*E-mail addresses*: kshama003@gmail.com (K. Balapure), bhattnikhil2114@gmail. com (N. Bhatt), datta\_madamwar@yahoo.com (D. Madamwar).

(Wang et al., 2014). Anaerobic metabolic function facilitates reductive breakdown of azo dye molecules by cleaving the azo bond to the corresponding colorless aromatic amines, which, although resist further anaerobic degradation, are reported to be well amenable for aerobic degradation (Chan et al., 2009). In aerobic condition these amines could be mineralized by non-specific enzymes through hydroxylation and ring-fission of aromatic compounds (Wang et al., 2014). Earlier studies showed that many different types of fixed film and suspended growth systems have been used for degradation of textile wastewater through anaerobicaerobic systems (Kapdan and Oztekin, 2006; Chan et al., 2009; Senthilkumar et al., 2011). However, fixed film systems offer several other advantages in textile wastewater treatment when compared with suspended growth processes, such as handling convenience, increased process stability, little residual sludge, high biomass retention, ease of use in small scale treatment, more energy efficient and capacity to handle various shock loads (Balapure et al., 2015).

Keeping this in mind, the present study was focused on the treatment of textile industrial wastewater using anaerobic treatment followed by microaerophilic fixed film reactor using pumice stone as a bedding material. Microaerophilic fixed film reactor combines advantages of the aerobic reactor (i.e. short hydraulic retention time (HRT), high biomass concentration, high specific surface area) and the anaerobic process (low quantities of waste, biological solids) (Laquidara et al., 1986). Thus, the sequential treatment ensures that, aromatic amines generated under anaerobic phase are further degraded and mineralized under microaerophilic phase which was seeded with enriched consortium BDN.

The efficacy of microaerophilic fixed film reactor seeded with bacterial consortium BDN for mineralization of simulated wastewater containing mixture of six dyes (dye concentration of 300 mg  $l^{-1}$ ) have been already reported in our earlier study (Balapure et al., 2015). However, the microaerophilic fixed film reactor treatment was unable to provide complete degradation of industrial textile wastewater, due to the presence of high amount of COD and color concentration in textile industrial effluent as compared to simulated textile wastewater. Thus, anaerobic step was added before microaerophilic reactor for complete degradation of textile wastewater.

The biodegradation of textile industrial wastewater having 10,000 mg l<sup>-1</sup> COD and 3340 Pt–Co color was carried out through sequential anaerobic-microaerophilic reactor under different organic loading rate. The effect of organic loading rate on COD, BOD, TS, TDS, TSS, TVS, chloride reduction etc. were determined to verify treatment efficiency. The degradation of textile wastewater and its metabolites in both anaerobic and microaerophilic reactors were detected by UV–vis, (Ultraviolet–Visible spectroscopy), FTIR (Fourier transformed infrared spectroscopy), <sup>1</sup>H NMR (<sup>1</sup>H Nuclear magnetic resonance spectrometry) and GC–MS (Gas chromatography-mass spectrometry) analysis. The toxicity of the textile industrial wastewater before and after sequential treatment was also studied by assessing its phytotoxicity.

# 2. Material and methods

#### 2.1. Sampling, characterization of industrial textile wastewater

Textile industrial wastewater was collected from the local textile industry, near Naroda G.I.D.C, Ahmedabad, Gujarat, India and stored at 4 °C till further use. It is worth to mention here that the dyeing process of this factory is continuous and utilizes many different types of azo dyes. The wastewater was characterized as per Standard Methods for The Examination of Water and Wastewater (APHA, 2012). Mean characteristics of the wastewater are presented in Table 1. Physico-chemical analysis of the wastewater was carried out for two months to cover variations in wastewater characteristics.

## 2.2. Experimental set-up

The laboratory scale sequential anaerobic - microaerophilic fixed film reactors were used in the experiment. Both the reactors were constructed using glass column. The anaerobic fixed film reactor with a working volume of 1.5 l, was used with following specifications: reactor inner diameter 5.3 cm; reactor height 122 cm; media height 93 cm; total volume (without bedding material) 3.0 l. Microaerophilic fixed film reactor with a working volume of 750 ml having following specifications: reactor inner diameter 2.5 cm; reactor height 60 cm; media height 45 cm; total volume (without bedding material) 1.5 l. was used. The reactors were packed with uniform pieces (~119 mm<sup>3</sup>) of pumice stone (1 kg in anaerobic reactor and 550 g in a microaerophilic fixed film reactor). Anaerobic reactor was completely restricted from air supply to avoid gaseous exchange to maintain anaerobic conditions. In microaerophilic reactor, dissolved oxygen concentration was maintained in the range of  $0.06-0.08 \text{ mg } \text{l}^{-1}$  (Keharia and Madamwar, 2003). The hydraulic retention times (HRT) of the bioreactor were varied by changing the flow rate of the feed to the bioreactor. Textile industrial wastewater was fed into the anaerobic fixed film reactor at the required rate using a peristaltic pump (Gilson Miniplus 3, France). The effluent of the anaerobic fixed film reactor was used as the influent of the microaerophilic fixed film reactor. The abiotic and biotic control experiments were simultaneously performed having same reactor dimension parameters under similar conditions.

# 2.3. Inoculum development for bioreactors

# 2.3.1. Seed inoculum for upflow anaerobic fixed film reactor

Cattle dung slurry (3.2% w/v) was used as a source of anaerobic bacteria for the development of biofilm in the anaerobic fixed film reactor. Cattle dung is a cheap and abundant source, having good, abundant source of anaerobic microorganisms. Moreover, it is also known to degrade pollutants or transform pollutants into less toxic substances. Keharia and Madamwar (2003) had used cattle dung slurry as an initial inoculum for the enrichment of strict anaerobes in the anaerobic fixed film reactor.

#### 2.3.2. Seed inoculum for microaerophilic fixed film reactor

Bacterial consortium BDN was used as a seed culture for the development of biofilm in the microaerophilic fixed film reactor. Bacterial consortium BDN was developed by culture enrichment technique, using Bushnell Hass Medium (BHM) amended with 100 mg  $l^{-1}$  model dye (Reactive Blue 160) and 0.5% yeast extract

Physico-chemical characterization of textile industrial wastewater.

Parameters	Concentration
рН	7.5 ± 0.3
Color (Pt–Co)	3340 ± 25.7
$COD (mg l^{-1})$	$10,000 \pm 34.3$
$BOD_5 (mg l^{-1})$	3330 ± 24.2
Total alkalinity (mg l <sup>-1</sup> )	3950 ± 31.6
Total solids (mg l <sup>-1</sup> )	4220 ± 25.4
Total suspended solid (mg $l^{-1}$ )	1510 ± 20.8
Total dissolved solids (mg $l^{-1}$ )	$1960 \pm 19.8$
Chloride (mg l <sup>-1</sup> )	$2400 \pm 14.8$
Phosphate (mg $l^{-1}$ )	$650 \pm 22.5$
Sulphate (mg l <sup>-1</sup> )	930 ± 29.5

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