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# Reactive black-5 azo dye treatment in suspended and attach growth sequencing batch bioreactor using different co-substrates



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#### A R T I C L E I N F O

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### ABSTRACT

Treatment of textile wastewater is a big challenge because of diverse chemical composition, high chemical strength and color of the wastewater. In the present study, treatment of wastewater containing reactive black-5 azo dye was studied in anaerobic sequencing batch bioreactor (SBBR) using mixed liquor suspended solids (MLSS) from suspended and attach growth bioreactors. MLSS at concentration of 1000 mg/L and reactive black-5 azo dye at 100 mg/L were used. A culture  $(10^8-10^9 \text{ CFU/ml})$  of pre-isolated bacterial strains (*Psychrobacter alimentarius* KS23 and *Staphylococcus equorum* KS26)) capable of degrading azo dyes in mineral salt medium was used to accelerate the treatment process in bioreactor. Different combinations of sludge, culture and dye were used for treatment using different co-substrates. About 85% COD removal was achieved by consortium (MLSS + KS23 + KS26) after 24 h in attach growth bioreactor compared to 85% color removal in suspended bioreactor. Addition of bacterial culture (20%, v/v) to the bioreactor could enhance the rate of color removal. This study suggests that biotreatment of wastewater containing textile dyes can be achieved more efficiently in the attach growth bioreactor using yeast extract as a co-substrate and MLSS augmented with dye-degrading bacterial strains.

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### 1. Introduction

Textile industry is one of the biggest water and chemical consuming industry releasing 20–50% dye in spend dye batch, resulting in colored and high strength wastewater (Lewis, 1999; Senthilkumar et al., 2011). Among the different classes of dyes, azodyes are mostly used and it is estimated that in textile industry up to 70% of all dyes applied are from azo dye group (-N=N-) (Zollinger, 1987; Zhang et al., 2004; Hsueh and Chen, 2008). The dye polluted wastewater is a serious environmental concern because of its carcinogenic nature, toxic byproducts, high oxygen demand, reduction of visibility in water bodies and inhibition in photosynthetic activity of aquatic plants (Weisburger, 2002; Hsueh and Chen, 2008). Such wastewater must be treated prior to its discharge into wastewater streams to prevent contamination of water resources and environmental deterioration.

Previously, several studies have shown that azo dyes can be removed by physical and chemical means, but physicochemical processes are considered costly and also produce huge amount of sludge (Jia et al., 1999; Balcioglu and Arslan, 2001; Ghoreishi et al., 2003). Similarly, in biological process aerobic treatment cannot effectively remove color of azo dyes because the azo bond is stable to aerobic biodegradation (Rai et al., 2005). However, anaerobic decolorization with digested sludge has proven its effectiveness (Banat et al., 1996; Sentillkumar et al., 2011). Some of the common anaerobic techniques used for the treatment of wastewater are anaerobic granular sludge (Razo-Flores et al., 1997), up-flow anaerobic fixed bed reactor (Sandhya and Swaminathan, 2006) and anaerobic biofilm membrane bioreactor (Spagni et al., 2007). All these systems are efficient but technically complex. Sequencing batch bioreactor with suspended and attach film suspended biocarriers is one of the easiest systems in treating high strength colored wastewater (Guo et al., 2009; Jamal Khan et al., 2010; Saba et al., 2011). The use of microbial cultures carrying efficient dye-degrading microbial strains could be an effective strategy for the treatment of dye-containing industrial wastewaters. A consortium of microorganisms could probably be more effective due to their tolerance to a wide range of pH and temperature, and ability to utilize a variety of feedstock under varying organic loading rate as compared to pure cultures (Watanabe and Bakar, 2000; Forgasc et al., 2004).

In the present study, a typical reactive black-5 azo dye was treated in sequencing batch bioreactor with microbial consortium

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of domestic wastewater and pre-isolated dye-degrading bacterial strains. An innovative aspect of the study is to show the efficiency of decolorization and COD removal by provision of glucose and yeast as co-substrates and to compare efficiency of the systems under different hydraulic retention times. MLSS was grown in SBBR and small batches of dye degradation under static conditions were established with MLSS taken from SBBR.

### 2. Materials and methods

#### 2.1. Microbial culture

Activated sludge was obtained from I-9 Wastewater Treatment Plant of Islamabad, Pakistan. Two previously isolated bacterial strains, namely Psychrobacter alimentrius KS23 and Staphylococcus equorum KS26, capable of decolorizing reactive azo dyes efficiently in liquid mineral salt medium (Khalid et al., 2012) were used to enhance the rate of color and COD removal. The bacterial culture was prepared in 250 ml conical flasks containing mineral salt medium (Khalid et al., 2008) and incubated at 30 °C for 48 h. Uniform cell density  $(10^8-10^9 \text{ CFU/ml})$  of the culture was maintained prior to its use for biotreatment studies.

#### 2.2. Experimental setup

In this study, two bench scale bioreactors (4 L capacity) such as suspended and attach growth as shown in Fig. 1 were used for the acclimatization of facultative mixed liquor suspended solids (MLSS). Temperature of the bioreactors was maintained at  $25 \pm 1$  °C thermostatic conditions. Activated sludge collected from wastewater treatment plant was acclimatized with synthetic wastewater for a period of 40 days. For biofilm maintenance, polyurethane cubes having density of 30 kg/m<sup>3</sup> and 10% (v/v) of the reactor volume were used as the moving media. It is commercially known as Unifoam (United Foam Industries Pvt. Ltd., Pakistan). Sponge cube of 1-cm<sup>3</sup> was selected as optimized earlier by Guo et al. (2010). The sludge acclimatization was ensured by the COD removal efficiency of 95–96% and biological growth of microorganisms from 2000 to 5000 mg VSS/L. After acclimatization under aerobic-

#### Table 1

Composition of dye treating batch system.

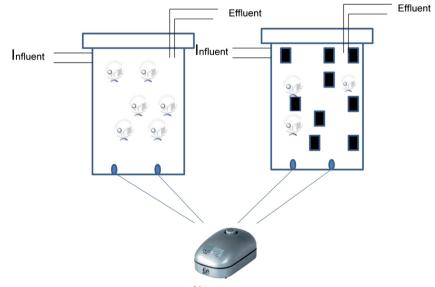
Parameters	Values
MLSS (mg/L)	1000
Dye	Reactive black-5
Dye concentration (mg/L)	100
Microbial consortia	20% solution
Microbial species	Psychrobacter alimentariuss (KS23)
	Staphylococcus equorum (KS26)
Co-substrates	C1 (mineral salts plus yeast extract)
	C2 (mineral salts plus glucose)
	C3 (Yeast extract only)
рН	$7\pm0.5$
COD (mg/L)	>1000
Hydraulic retention time HRT (h)	24
Reaction conditions	Anaerobic/static

anoxic-aerobic cyclic conditions, the sludge was transferred to their respective batch reactors under completely anaerobic static condition. Every batch of the dye treatment contained 100 mg/L concentration of reactive black-5 azo dye, 1000 mg of MLSS, 20% (v/ v) bacterial culture and different co-substrates coded as C1 containing yeast extract with mineral salts (Khalid et al., 2008), C2 containing glucose in synthetic wastewater composition (Jamal Khan et al., 2010) and C3 having only yeast extract. Observations were recorded for color and COD removals. Table 1 shows the parameters and their values maintained in batch systems.

Each experimental study was carried out at least for 48 h hydraulic retention time (HRTs) and repeated in triplicates. Sludge age was adjusted to 10 days by removing certain amount of sludge daily.

#### 2.3. Analytical methods

COD was determined using closed reflux colorimetric method (APHA 2005). Soluble COD samples were obtained by settling the sludge sample for 30 min and filtering the sample with 0.45  $\mu$ m filter (GF/C, Whatman, USA). MLSS, mixed liquor volatile suspended solids (MLVSS), sludge volume index and sludge density index were analyzed according to standard methods (APHA 2005).



Air compressor

Fig. 1. Schematics of bench scale reactor for MLSS acclimatization.

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