



Short Communication

Biodegradation of azo dyes and dyes present in textile wastewaters using *Bacillus* sp. az28, obtained from industrial effluents

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ABSTRACT

The bacterial isolate *Bacillus* sp. AZ28, obtained from industrial effluent, demonstrates a great capacity to degrade various azo dyes (methyl orange (MO), magneson I (MI), novacron dark blue (NDB), and novacron red FN 3GF (NRF3)), and azo dye-containing textile effluent (TE). The degradation was evident by decolorization of dyes, and the decolorization efficiency of 84-95% was achieved within 14–72 h under optimum conditions, such as 37°C, pH 7, inoculation size 8%, 1% glucose, and 1% beef extract. The extent of decolorization of individual dye was determined by UV–Vis spectroscopy, and products of biodegradation were analyzed by FTIR spectroscopy and TLC analyses. Chemical analysis showed that the COD and BOD values were significantly reduced after treatment. Thus, the biodegradation ability under mild conditions suggests that *Bacillus* sp. AZ28 has potential in textile effluent treatment.

Azo dyes are widely used in textile and tannery industries, but conventional technologies cannot degrade/mineralize the dyes that remained unused after the industrial processes (Liao et al., 2013; Kanagaraj et al., 2012; Singh et al., 2015). As a result, improper discharge of highly colored effluent causes damage to the aquatic environment due to ecotoxicological effects caused by reduced light penetration and photosynthesis. Therefore, research on the degradation of azo dyes is of recent interest (Wu et al., 2012). Physical and chemical methods were previously used for the treatment of azo dye-containing industrial effluents (Wang et al., 2009). These methods are not cost-effective;

besides, they generate secondary pollutants due to the use of excessive chemicals (Tony et al., 2009). Recently, environment-friendly biological methods are widely used because they are less costly and produce less sludge (Gopinath et al., 2009; Barapatre et al., 2017). However, due to the application of new dyes in textile industries and their environmental impact (Hossen et al., 2019), further studies are required to find suitable and effective methods for the treatment of effluents. In this communication, we report the biodegradation studies of two azo dyes (MO and MI), two azo dyes (NDB and NRF3) used in textile industries, and textile effluent (TE) by a bacterial strain *Bacillus* sp., isolated from local textile effluent.

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The azo dye degradation was studied by UV-Vis and FTIR spectroscopy, TLC analyses, and COD and BOD measurements. The bacterial strain was isolated from industrial effluent and identified as *Bacillus* sp. AZ28 (Accession no. LC202628) was based on the 16S rDNA sequence reported previously (Hossen et al., 2019). Eight percent (v/v) inoculum of *Bacillus* sp. AZ28 was separately inoculated into solutions containing each azo dye – MO (50 ppm), MI (100 ppm), NDB (100 ppm), NRF3 (100 ppm), and TE in 50 mL conical flasks. The degradations were carried out under static conditions at 37°C, and 6 mL solution from each sample was withdrawn at every 2 h interval up to 14 h to observe the decolorization of MO, MI, and NRF3. Samples of NDB and TE were withdrawn at every 24 h interval up to 72 h. An aliquot of the sample was centrifuged at 5000 rpm for 15 minutes to remove biomass. The percentage of decolorization of the supernatant was determined by measuring the decrease in the absorbance at λ_{\max} for each dye (463 nm, 451 nm, 598 nm, 503 nm, and 492 nm for MO, MI, NDB, NRF3, and TE, respectively) at definite time intervals during the decolorization process. The percentage of decolorization as a function of incubation time was determined by measuring the absorbance of the dye solution at the respective λ_{\max} before and after incubation. The remaining aliquot of the sample was used for determining the biomass by

measuring absorbance at 600 nm (Olukanni et al., 2010).

Eight percent (v/v) inoculum of *Bacillus* sp. AZ28 decolorized MO, MI, NRF3 up to 92%, 84%, and 95%, respectively, after 14 h of incubation (Table 1). However, the decolorization of NDB and TE by the same bacterium was 92% and 95% after 24 h and 72 h of incubation, respectively (Table 1). The decolorization of all dyes increases with the increasing biomass of *Bacillus* sp. AZ28 (Fig. 1). MO and MI required only 1.8 g/L and 3.1 g/L biomass for 92% and 84% decolorization, respectively, whereas NRF3, NDB, and TE require 6.1 g/L, 8.1 g/L, and 7.9 g/L biomass for 95%, 95%, and 92% decolorization, respectively. Previous studies on a different type of azo dye using *Proteus mirabilis* LAG also showed that the decolorization increased with increasing biomass (Olukanni et al., 2010).

The COD and BOD of each parent dye solution and degraded solutions were measured using the standard $K_2Cr_2O_7$ method (Kalyani et al., 2009) and the Winkler method (Ahmed, 2001), respectively. The COD and BOD values of each dye were significantly reduced after the degradation (Table 1). The highest reduction of COD (93%) and BOD (94%) values were observed in the degraded solution of NRF3. Gurulakshmi et al. (2008) also observed the reduction of BOD (62%) and COD (76%) after the decolorization of congo red by *Bacillus* sp.

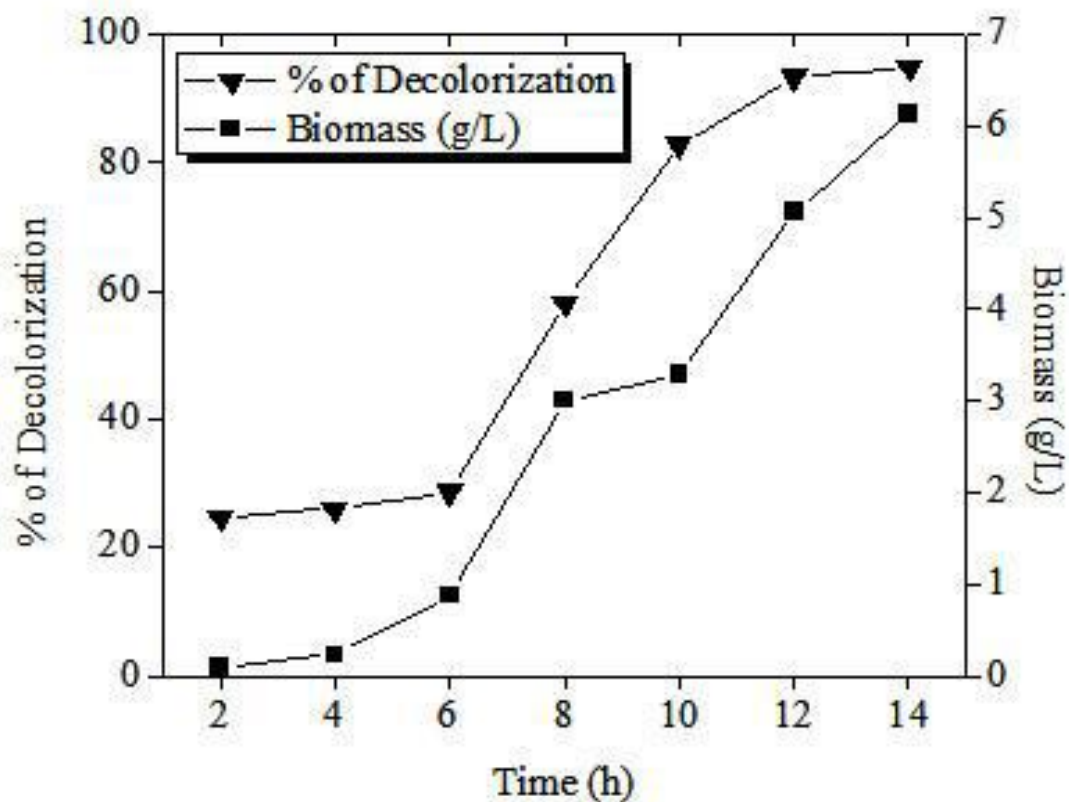


Fig. 1. Decolorization of NRF3 and biomass growth during 14 h incubation.

Table 1. Decolorization and removal of COD and BOD of azo dyes.

Name of Dye	Concentration (ppm)	Incubation time (hour)	Decolorization (%)	COD removal (%)	BOD removal (%)
MO	50	14	92	39	38
MI	100	14	84	23	25
NRF3	100	14	95	93	94
NDB	100	72	95	22	65
TE	-	24	92	39	47

Decolorization studies on all dyes at various pH and temperatures demonstrated that pH 7.0 and temperature at 37°C were the optimum conditions. Previous studies on congo red by *Bacillus* sp. support our finding (Gopinath et al., 2009). Degradation of dyes by *Bacillus* sp. AZ28 was increased with the increase of inoculum size up to 8%. Studies on the effect of nutrients showed that the bacterium showed better decolorizing capability on MO (90%), MI (86%), NRF3 (93%), NDB (96%), and TE (91%) with glucose (compared to dextrin, lactose, and galactose) as the carbon source, and with beef extract (compared to peptone and yeast extract) as the nitrogen source, suggesting the dependence of decolorization on the nutrients. Similarly, *Bacillus cereus* strain HJ-1 showed better performance on reactive black B when glucose and yeast extract were used as carbon source and nitrogen source, respectively (Liao et al., 2013).

To understand the real cause of decolorization, we analyzed the supernatant by TLC and FTIR spectroscopy. Ethyl acetate solutions of parent dyes and degraded residue were spotted on silica TLC plates. A mixture of dichloromethane and n-hexane (3/7) was used as a mobile phase. The resolved chromatogram was kept in an iodine chamber to detect spots (Kanagaraj et al., 2012). In the TLC plates, multiple spots were observed for the degraded residue, which was absent in pure dyes, suggested the formation of metabolic intermediates (Fig. 2). Kanagaraj et al. (2012) also used TLC analysis to study the formation of metabolites intermediates of azo dyes.

The degradation of dyes was also studied by observing characteristic IR peaks of the functional groups. The parent azo dyes and textile effluent generally contain -N=N- (present in all dyes), -N=O, -N-O, -S=O, -OH functional groups, which gave characteristic peaks in the IR spectra. However, these peaks

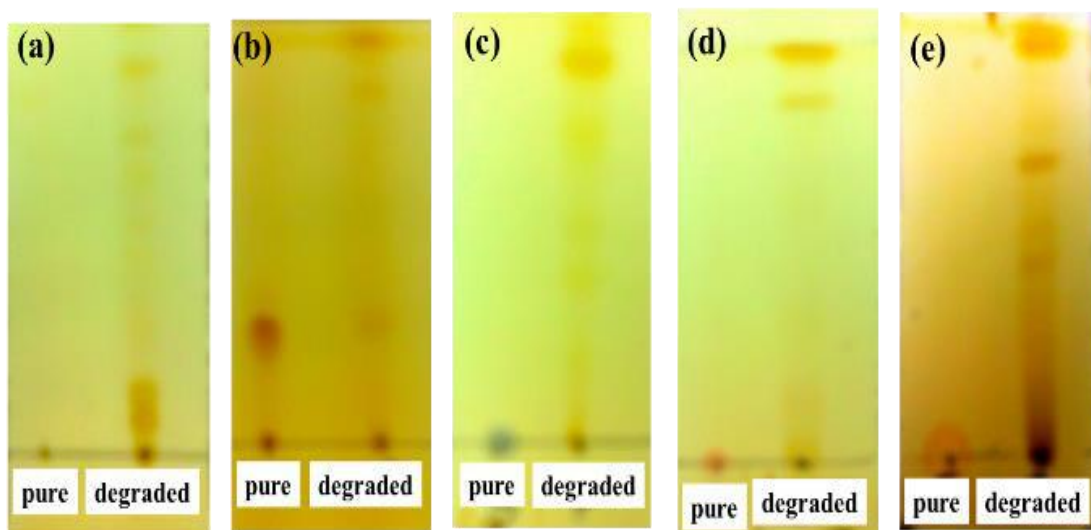


Fig. 2. Spots of pure and degraded (a) MO, (b) MI, (c) NDB, (d) NRF3, and (e) TE on silica.

disappeared or changed, and new peaks were observed due to the decomposition of the -N=N- group of the dyes. For example, the IR spectrum of megneson I displayed absorption peaks at 1642 and 1595 cm^{-1} for the aromatic C=C stretch, 1557 and 1518 cm^{-1} for -N=N- stretch, 1233 cm^{-1} for C-N stretch, 1475 and 1341 cm^{-1} for symmetric and asymmetric modes of NO_2 , 1209 and 1109 cm^{-1} for in-plane and out of plane bending of the -OH. After treatment, most of the peaks disappeared, and new peaks were observed at 3400 cm^{-1} (for N-H and O-H stretch), 2920 and 2848 cm^{-1} (for aliphatic C-H stretch), and 1616, 1567, and 1541 cm^{-1} (for C=C stretch). Therefore, the disappearance of the characteristic peaks of a particular azo dye after the treatment with the bacterial organism demonstrates the degradation of these dyes (Barapatre et al., 2017).

In summary, *Bacillus* sp. AZ28, isolated from textile effluent, demonstrated azo dye degradation capability that can be used in designing new biological methods in the treatment of textile effluents. The degradation efficiency of *Bacillus* sp. AZ28 is dependent on the judicious choice of physicochemical parameters: pH, temperature, duration of treatment, carbon/nitrogen sources, and biomass quantities. Analyses of the metabolites by FTIR spectroscopy and TLC suggest that bacterial decolorization is associated with dye degradation.

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