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Decolorization and Degradation of Reactive Blue Dye Used in Jute and Textile Industries by a Newly Isolated *Bacillus* sp.

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ABSTRACT

For biodegradation of reactive dyes used in jute and textile industries, bacteria were isolated from a dyeing mill effluent. Bacteria having a remarkable ability to decolorize and degrade reactive dye were screened by using dye Reactive Dark Blue WR (RDB-WR). Cultural, morphological and biochemical characteristics were observed and based on these seven isolates having higher decolorizing capability was identified. Among these isolates, one of the prominent dye decolorizing isolates *Bacillus* sp. was taken for decolorization study. Under different physicochemical conditions, decolorization and degradation capabilities of *Bacillus* sp. were optimized by using RDB-WR, a dye commonly used in the jute and textile industries. This bacterium decolorized and grew well up to 500 mg L^{-1} of RDB-WR. *Bacillus sp*. showed significant decolorization approximately 86% at 200 mg L^{-1} of RDB-WR after 96 h of incubation. Optimum degradation of dye was achieved at 37 °C. Maximum decolorization was observed at pH 7.0 under static conditions. The study confirmed the potential of *Bacillus* in the biodegradation of Reactive Dark Blue WR. This bacterial isolate might be prospective in the biological treatment of dyeing mill effluents due to the high extent of decolorization.

Keywords: Bacterial isolate, Decolorization, Jute, Biodegradation, RDB, Textile industry, and *Bacillus* sp.

1. INTRODUCTION:

Environmental pollution is one of the major concerns of today's world. Due to rapid industrialization and urbanization large amount of wastes are generated and discharged into the environment and causing major pollution problem. Among many pollutants, effluents from dyeing industries are the major source of aquatic environmental pollution. Textile, pharmaceutical, cosmetic, jute, paper and food industries use synthetic dyes widely (Pandey *et al*., 2007; Kant, 2012). For complex aromatic molecular structures, synthetic dyes are more recalcitant to biodegradation (Aksu, 2005; Dellamatrice *et al*., 2017). Textile and jute industries use around 10,000 different dyes, pigments and worldwide production is over 7×10^5 tons per annum (Aksu and Tezer, 2005; Daneshvar *et al*., 2007; Celia and Suruthi, 2016). There is a tremendous increase in dye utilization for rapid industrialization and man's demand for color (Mohan *et al*., 2002). Various color shades, easier application, high wet fastness, brilliant colors formation and consumption of minimal energy are the main reasons for extensive usage of reactive

dyes in the textile and jute industries (Shah *et al*., 2013). Azo, anthraquinone and phthalocyanine are the common groups of reactive dyes (Axelsson *et al*., 2006) and these are mostly toxic, mutagenic and carcinogenic (Acuner and Dilek, 2004; Rauf and Ashraf, 2012; Stiborova *et al*., 2013). Aquatic environment can be damaged due to inappropriate discharge of reactive dye containing effluents. Aromatics, chlorides and metals of reactive dyes may be harmful for some aquatic life and photosynthesis in aquatic phototrophs may be affected severely for reduced penetration of light (Celia and Suruthi, 2016). For high tinctorial value less than 1 ppm of the reactive dye is needed for color production (Gupta *et al*., 2003). Adsorption, coagulation–flocculation, oxidation and electrochemical are the physical and chemical methods normally used for removing dye from wastewater. But high-energy costs, by-products formation and production of high-sludge are the main limitations of these methods (Sarioglu *et al*., 2007; Celia and Suruthi, 2016). In contrast, bio-processing can surmount these drawback for being cost saving and environment friendly (Kurade *et al*., 2017).

Recent studies have focused on using microorganisms for degrading dyes from effluents (Chen *et al*., 2003; Acuner and Dilek, 2004; Mishra and Malik, 2014; Shen *et al*., 2015; Kurade *et al*., 2017; Veena *et al*., 2019). Bacteria, fungi, yeast and algae can decolorize and degrade wide range of dyes (Ayed *et al*., 2010; Kabra *et al*., 2011; Patel *et al*., 2013; Saratale *et al*., 2013; Veena *et al*., 2019) and many reactive dyes can be quickly degraded and even mineralized completely by bacteria under appropriate conditions (Chen *et al*., 2003; Asad *et al*., 2007; Jadhav *et al*., 2011; Kurade *et al*., 2012; Barapatre *et al*., 2017). In decolorization process, intermediate metabolites like aromatic amines are produced which can be degraded by the hydroxylase and oxygenase generated by bacteria (Pandey *et al*., 2007; Wanyonyi *et al*., 2017).

Jute is the golden fibre of Bangladesh. Jute and jute products have become second exporting goods of Bangladesh. Moreover, Bangladesh has emerged as one of the largest garment-manufacturing nations in the world. It has become the largest sector of Bangladesh in terms of foreign currency earnings and employment generation (Farhana *et al*., 2015, Shuchismita and Ashraful, 2015). Huge amount of reactive dyes are used in manufacturing processes in textile and jute industries and are discharged through effluents with-out any treatment (Chindah *et al*., 2004). In Bangla-desh the physicochemical parameters of the dis-charged effluents are much higher than the standard value recommended by Department of Environment (Shuchismita and Ashraful, 2015). Reactive dyes present in surface and subsurface water make them aesthetically obnoxious. Human health hazards resul-ting in diseases, viz. dermatitis, mucous membrane, perforation of nasal septum and respiratory tract irritation, toxicological effects as well as allergy are caused by reactive dyes (Islam *et al*., 2011; Yadav, 2014; Rovira and Domingo, 2019).

Textile and jute effluents impart a chemical load to the environment and make the environment quite unacceptable. People living near dyeing industries are now being in danger for environmental polution (Sultana *et al*., 2009). Therefore, a sustainable bio-process is very essential to remedy the toxicity caused by the reactive dyes present in the untreated industrial effluents. For this reason, finding reactive dyes-degrading bacterial isolates from the native environment is quite important. Bacteria present in the jute and textile effluents might have reactive dye degrading ability. A number of studies have revealed absorption and degradation of dyes by microorganisms (Mishra and Malik, 2014; Shen *et al*., 2015; Kurade *et al*., 2017; Veena *et al*., 2019), further studies are essential to develop biotechnology to detoxify and degrade the reactive dyes in effluents generated from jute and textile industries. In this study, bacteria were isolated and identified from dyeing mill effluent. Different physicochemical parameters were optimized for decolorization of Reactive Dark Blue WR (RDB-WR), one of the reactive dyes used in jute and textile industries.

2. MATERIALS AND METHODS:

2.1. Chemicals - A reactive dye-namely Reactive Dark Blue WR (RDB-WR), used in jute and textile industries was collected from a dyeing industry located at Chittagong, Bangladesh. All other chemicals were purchased from Sigma Aldrich, India unless noted.

2.2. Sample collection and Estimation of total viable bacterial count - Samples were collected in sterile vials from effluent of dyeing industry located at Chittagong, Bangladesh. Transportation of samples was done immediately to the laboratory and stored at 4°C before used in the experimental purpose. Total viable bacterial count (TVBC) in these samples was enumerated as descrived by Azad *et al*. (2009). The unused samples were preserved in the same condition for repeatation of the experiment.

2.3. Isolation, screening and identification of dye decolorizing bacterial isolates - Samples collected from effluent were diluted up to 10^4 times and cultured separately on the nutrient agar plate as described by Hossen *et al*. (2019). 14 bacterial colonies from nutrient agar plates were selected randomly for pure culture. Screening of these isolates for decolorization capability was carried out for 7 days using the reactive dye. For screening the bacterial isolates, having the dye degradation capability, one loop full of each bacterial isolate was inoculated in 10 ml sterile nutrient (glucose, 1 gm L^{-1}; beef extract, 1 gm L^{-1}; peptone, 1 gm L^{-1} and yeast extract, 1 gm L^{-1}, pH 7.0) broth containing 200 mg L^{-1} of dye in the test tubes. Usually 100-300 mg L^{-1} dye is used for screening dye decolorizing bacteria (Lalnunhlimi and Krishnaswamy, 2016). Dye decolorizing isolates were selected and identified based on cultural, morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology (Staley *et al*., 2001).

2.4. Measurement of decolorization extent - A 5% (v/v) freshly cultured bacterial inoculum was inoculated into dye containing sterile nutrient broth in 50 ml conical flask. The experiment was carried out at 37 °C under static condition for a period as noted. Culture (2 ml) was collected every 24 h and centrifuged at 8000 rpm for 10 min to remove bacterial cells. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 635 nm (λ_{max}) using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). Decolorization extent was calculated using the following equation:

$$\text{Decolorization extent } (\%) = \frac{OD1 - ODt}{OD1} \times 100$$

Where, OD1 refers to the initial absorbance before Decolorization; ODt refers to the absorbance after decolorization.

2.5. Effects of different physicochemical parameters on dye decolorization of RDB-WR - Optimization of different physicochemical parameters RDB-WR dye were performed. From the bacteria, one isolate from the genus, *Bacillus* (isolate 12) (Uddin *et al*., 2017), which was prominent decolorizer during screening of RDB-WR was selected. Media containing 50, 100, 200, 500 and 1000 mgL^{-1} dye were subjected to the decolorization process to study the effects of initial dye concentration. The experiment was conducted at 37 °C and pH 7.0. To estimate the optimum temper-ature, the study was done at 30, 37 and 45 °C. The initial pH of the media was 7.0. To study the effects of different pH on the decolorization, pH of the media were adjusted to 5.0 to 9.0. The experiment was carried out at 37 °C.

2.6. Statistical analysis - Student's *t* test was used for statistical analysis. A *P* value of <0.05 was considered as statistically significant. Data were presented as the means of repeated experiments (n=5).

3. RESULTS AND DISCUSSION:

3.1. Estimation of Bacterial Load - Total viable bacterial count (TVBC) of receiving water provides valuable statistics in monitoring various types of pollutants. The heterotrophic bacteria were counted by the spread-plate technique by using nutrient agar. Total viable bacteria found in the effluent were 5×10^2 CFU ml^{-1}. TVBC of normal pond water (2×10^6) was also enumerated (**Table 1**). Data reported herein suggested that this effluent may be toxic to the growth and survival of normal microflora.

Table 1: Total viable bacterial count of different sources of sample.

Sample	CFU/ml
Dyeing mill effluent	5×10^2
Pond water	2×10^6

3.2. Isolation, screening and identification of dye decolorizing bacterial isolates - For investigating the ability to decolorize RDB-WR, fourteen bacterial isolates were selected randomly from the nutrient agar

plate. Screening for dye decolorization of these isolates was carried out in decolorizing nutrient broth for seven days by using the reactive dye (200 mgl^{-1}) (**Table 2**). Seven isolates that decolorized the dye were chosen for further study. Cultural, morphological and biochemical characteristics indicated that these seven isolates belonged to the bacterial genus of *Bacillus* (2 isolates), *Pseudomonas* (2 isolates), *Aeromonas* (1 isolate), *Alcaligenes* (1 isolate) and *Serattia* (1 isolate). The morphological and biochemical characteristics of these isolates are summarized in **Table 3**. We selected isolate 12 (*Bacillus* sp) that was prominent decolorizer of dye during screening for further study.

Table 2: Screening of dye degrading bacteria following incubation at 37°C for 170 h.

Dye	Observation period	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Reactive DB WR	24 h	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	48 h	-	-	-	-	-	-	-	+	-	-	-	+	+	-
	72 h	-	-	-	-	-	-	-	+	-	-	-	+	+	+
	96 h	-	-	+	-	-	-	-	+	-	-	-	+	+	+
	120 h	-	-	+	-	-	-	-	+	+	-	+	+	+	+
	144 h	-	-	+	-	-	-	-	+	+	-	+	+	+	+
	170 h	-	-	+	-	-	-	-	+	+	-	+	+	+	+

Table 3: Morphological and biochemical characteristics of bacterial isolate.

Morphological and biochemical tests	Bacterial isolate 12
Shape	Rod
Motility	+
Catalase production	+
Oxidase production	+
Gram staining	+
Indole test	-
Methyl red test Voges-Proskauer test	+ -
Citrate utilization	-
Triple Sugar Iron Agar test (Slant/Batt)	K/A
Maltose fermentation	+
Lactose fermentation	-
Glucose fermentation	+
Spore test	+
Bacterial genus	*Bacillus* sp.

Here, K/A = Red/Yellow

3.3. Effects of initial dye concentration on the decolorization of RDB-WR - Dye decolorization and degradation depend on initial dye concentration as it has strong inhibitory effects (Khehra *et al*., 2005; Kalme *et al*., 2007). For this reason, decolorization activity of *Bacillus* sp. was studied using different RDB-WR concentrations ranging from 50 to 1000 mg L^{-1} (**Fig 1**). The study showed that the decolorization decreased with an increase in RDB-WR concentration.

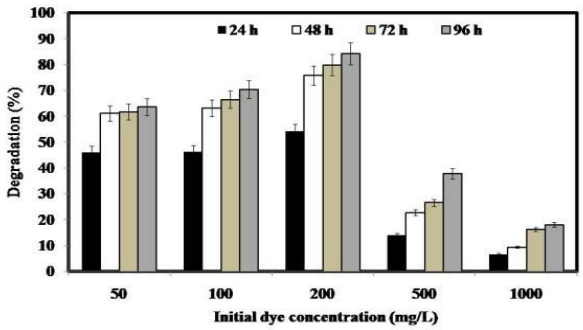

Fig 1: Effects of initial dye concentrations on decolorization of RDB-WR after 96 h of incubation with 5% inoculums. The initial pH in and the temperature were 7.0 and 37 °C, respectively.

It was observed that maximum decolorization by *Bacillus* sp. was approximately 85% with 200 mg L^{-1} RDB-WR (Ekhlas *et al*., 2014). Reports indicated the optimum decolorization by *Bacillus* species were (95-97%) when 200 mg L^{-1} of azo direct blue 151 and azo

direct red 31 were used (Lalnunhlimi and Krishnaswamy, 2016), Decolorization significantly decreased with 500 mg L^{-1} of RDB-WR and at 1000 mg L^{-1} of RDB-WR, decolorization was severely inhibited. Although bacterial growth was not notably decreased with 500 mg L^{-1} of RDB-WR, 30-40% of the bacterial growth was inhibited by 1000 mg L^{-1} of RDB-WR in nutrient broth (data not shown).

3.4. Effects of temperature on the decolorization of RDB-WR - Variation in temperature decreases or increases the enzyme activity responsible for dye decolorization and degradation. Maximum decolorization by *Bacillus* sp. was obtained at 37 °C, although a significant decolorization occurred at 30 °C (**Fig 2**). The optimum decolorization temperature for RDB-WR by *Bacillus* was in conformity with *Pseudomonas* sp. decolorization of brilliant green, malachite green, fast green, congo red and methylene blue (Mali *et al*., 2000), *A. faecalis* PMS-1 decolorization of reactive orange 13 (Shah *et al*., 2012) and *Bacillus subtilis* decolorization of fast red (Mona and Yusef, 2008).

However, the decolorization was strongly inhibited at 45°C, which might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization (Çetin and Dönmez, 2006). This result indicated that 30-37°C might be the best temperature for decolorization activity of this bacterial isolate.

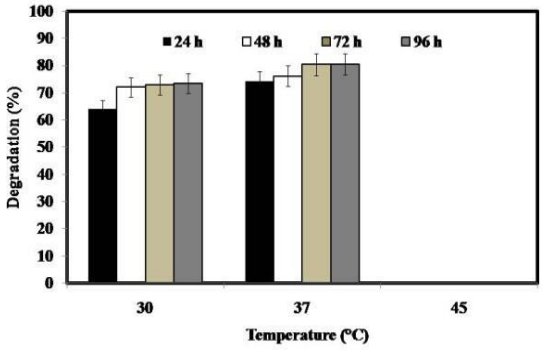

Fig 2: Effects of temperature on decolorization of RDB-WR after 96 h of incubation with 5% inoculum. The initial dye concentration and initial pH were adjusted to 200 mgL^{-1} and 7.0, respectively.

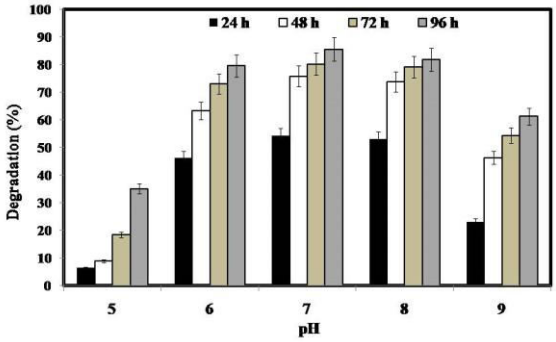

Fig 3: Effects of pH on decolorization of RDB-WR after 96 h of incubation with 5% inoculum. The initial dye concentration and the temperature were 200 mgL^{-1} and 37 °C, respectively.

3.5. Effects of pH on the decolorization of RDB-WR - The maximum level of decolorization of RDB-WR by *Bacillus* sp. was observed at pH 7.0 (**Fig 3**). However, high level of decolorization (82%) was also supported by pH 8.0. Moreover, significant levels of decolorization by this bacterial isolate were observed at pH 6.0 - 9.0. This result indicated that this isolate could decolorize RDB-WR within a wide range of pH, suggesting that this strain is potential organism for practical bio-treatment of dyeing mill effluents. The optimum pH for dye decolorization by *A. faecalis* PMS-1, *Bacillus* spp. and *Enterobacter* sp. is similar with the bacterial isolate used in the present study for RDB-WR decolorization (Shah *et al*., 2012; Lalnunhlimi and Krishnaswamy, 2016). The optimum pH for dye decolorization varies from acidic to alkaline condition (Saratale *et al*., 2011). Under alkaline condition reactive azo dyes bind to jute and cotton fibers by addition or substitution mechanisms so pH tolerance of decolorizing bacteria is very essential (Aksu and Donmez, 2003; Wang *et al*., 2013).

4. CONCLUSION:

Dye decolorizing bacteria were isolated from dyeing mill effluent in this experiment. 7 among these 14 bacterial isolates showed dye decolorizing ability. Physicochemical parameters for decolorizing RGB-WR were optimized by *Bacillus. Bacillus* sp. showed decolorizing activity through a degradation mechanism and this could tolerate up to 1000 mg L^{-1} of RGB-WR.

For high degradative and decolorizing ability against reactive dye used in the jute and textile industries, it can be anticipated that *Bacillus* sp. has a convenient application prospective in the transformation of dyeing mill effluents.

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6. CONFLICTS OF INTEREST:

The author (s) declared no potential conflicts of the interest with respect to the research, authorship and/or publication of this article

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