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Bacterial Degradation of Synthetic Dye by *Pseudomonas* sp. Obtained from Dyeing Mill Effluent

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ABSTRACT

Environmental pollution is one of the major concerns of today's world. Due to rapid industrialization and urbanization, a large number of wastes are generated and discharged into the environment and causing major pollution problems. For obtaining reactive dye decolorizing bacterial isolates, effluent samples were collected from a dyeing mill. From bacterial pure culture, 10 isolates were selected for screening. Screening of these isolates for the capability to decolorize and degrade reactive dye was performed in a nutrient broth medium containing reactive dye. 6 isolates among these bacterial isolates showed dye decolorizing ability within 120 hours of incubation. These isolates were further identified based on cultural, morphological, and biochemical characteristics. These characteristics indicated that these six bacterial isolates were distributed to the bacterial genus of *Bacillus* (2 isolates), *Pseudomonas* (2 isolates), *Aeromonas* (1 isolate), and *Alcaligenes* (1 isolate). For the study of dye decolorization and degradation, Reactive Dark Blue dye used in jute and textile industries was chosen. *Pseudomonas*, a prominent dye decolorizing isolate during screening, was taken for the optimization of different physicochemical parameters. This bacterium decolorized and grew well up to 500 mgL^{-1} of Reactive Blue dye. *Pseudomonas* sp. showed noteworthy decolorization of approximately 84% at 200 mgL^{-1} of dye concentration after 96 h of incubation. The optimum temperature for dye degradation was at 37 °C. The maximum level of decolorization for *Pseudomonas* sp. was observed at pH 8.0. This isolate showed better decolorization extent under static conditions rather than shaking conditions. This result indicated that the dye had been utilized by this bacterial isolate. It can be concluded that *Pseudomonas* is a prospective candidate in the biodegradation of Reactive Blue dye and might be useful in bioprocess technology used for the bioremediation of dyeing mill effluents.

Keywords: Bacteria, Decolorization, Biodegradation, Reactive dye, Dying mill, and *Pseudomonas* sp.

1. INTRODUCTION:

Effluent from dyeing industries is one of the main sources of aquatic environmental pollution among many pollutants. Different industries like textile, cosmetic, pharmaceutical, jute, food and paper mills utilize synthetic dyes extensively (Pandey *et al*., 2007; Kant, 2012). Usually dyes are not biodegraded easily for complex molecular structures (Dellamatrice *et al*., 2017). Total usages of various dyes and pigments in textile and jute industries is about 10,000 and total yearly production is over 7×10^5 tons (Daneshvar *et al*.,

2007; Celia and Suruthi, 2016). For rapid industrialization, the demand for dye is rising immensely (Mohan *et al*., 2002), and there is huge utilization of reactive dyes in textile and jute industries for high wet fastness, various color shades, brilliant colors, easier application and less energy consumption (Shah *et al*., 2013). Azo, anthraquinone and phthalocyanine are the most common type among the reactive dyes (Axelsson *et al*., 2006) and majority of these are toxic, mutagenic and carcinogenic (Stiborova *et al*., 2013). Inappropriate release of effluents containing reactive dyes has detrimental effects on the aquatic environment for metals, aromatics, chlorides, etc. present in dyes. This may extensively hamper photosynthesis for reduced penetration of light (Celia and Suruthi, 2016).

Different physico-chemical methods like adsorption, oxidation, coagulation-flocculation and electrochemical methods are generally used for the remediation of dye from dyeing mill effluents (Lin and Peng, 1994) but these have many demerits like high-sludge production, high cost and by-products formation (Celia and Suruthi, 2016). But bioprocessing can surmount these drawbacks for being cost effective and ecofriendly (Kurade *et al*., 2017). So development of effective process for removal of dyes from wastewater is badly needed.

Bioprocessing has gained growing interest as a feasible alternative owing to cost effectiveness, less sludge generation and environment-friendly nature. Bacteria, yeast, fungi and algae are competent for decolorizing various dyes (Ayed *et al*., 2010; Kabra *et al*., 2011; Patel *et al*., 2013; Saratale *et al*., 2013; Veena *et al*., 2019) and recent studies have given importance on using these microorganisms (Mishra and Malik, 2014; Shen *et al*., 2015; Kurade *et al*., 2017; Veena *et al*., 2019). Moreover, bacteria can not only degrade but also totally mineralize reactive dyes under suitable conditions (Jadhav *et al*., 2011; Kurade *et al*., 2012; Barapatre *et al*., 2017). Bacterial hydroxylase and oxygenase enzymes can degrade aromatic amines, intermediate metabolites produced in degradation of dyes and pigments (Pandey *et al*., 2007; Wanyonyi *et al*., 2017).

In Bangladesh, textile and jute sectors are two important sectors from agricultural, industrial, economic and commercial perspectives. Bangladesh is a prominent manufacturer and exporter of jute and jute diversified products. The export market is becoming attractive gradually as people around the world are more getting environmentally conscious. Moreover, Bangladesh is second in garment-manufacturing and this is the largest sector for earnings foreign currency and employment generation (Farhana *et al*., 2015; Shuchismita and Ashraful, 2015). These industries use huge amount of dyes and discharge wastewater without treatment (Chindah *et al*., 2004) and the parameters for physicochemical property are found much higher than the recommended value of Department of Environment (Shuchismita and Ashraful, 2015).

Reactive dyes present in subsurface water make them aesthetically obnoxious and health hazards like dermatitis, mucous membrane, perforation of nasal septum and respiratory tract irritation; toxicological effects are the results (Islam *et al*., 2011; Yadav, 2014; Rovira and Domingo, 2019). For the remediation of reactive dye generated toxicity, a bioprocess - that is effective and sustainable is needed and obtain-ning reactive dye-degrading bacteria from effluent is significant. Numerous studies have shown microbial dye decolorization through absorption and degradation (Mishra and Malik, 2014; Shen *et al*., 2015; Kurade *et al*., 2017; Veena *et al*., 2019), further studies are needed to develop biotechnological process for the degradation and detoxification of dye containing effluents.

Isolation of bacteria from effluent of untreated dyeing industry was performed in the present study. These dye decolorizing bacteria were further identified. Reactive Blue dye applied in dyeing purpose in jute and textile industries was used for optimizing various physicochemical parameters for decolorization.

2. MATERIALS AND METHODS:

2.1. Chemicals - For this study, a reactive dye-namely Reactive Dark Blue dye was collected from Four H Dyeing Industry located at Chittagong, Bangladesh. All other chemicals were bought from Sigma Aldrich, India.

2.2. Sample collection – For sample collection, in sterile vials were used. Samples were collected from

effluent of dyeing industry located at Chittagong, Bangladesh and transported to the laboratory as early as possible. These effluent samples were stored at 4°C for the experiment.

2.3. Isolation, screening and identification of dye decolorizing bacteria - Samples collected from dyeing mill effluent were serially diluted up to 10^4 times. These diluted samples were separately cultured as described by Hossen *et al*. (2019). Nutrient agar plates were used for this purpose. For getting pure culture, 10 colonies of bacterial isolates were selected randomly. Screening for dye decolorizing bacterial isolates was carried out for 7 days using the reactive dye as previously described (Hossen *et al*. 2020). Usually 100-300 mg L^{-1} dye concentration is used for decolorization study (Lalnunhlimi and Krishnaswamy, 2016). After screening, dye decolorizing bacteria were identified. Bacterial identification was done based on morphological, cultural and biochemical characteristics and according to Bergey's Manual of Systematic Bacteriology (Rahman *et al*., 2019; Staley *et al*., 2001).

2.4. Measurement of decolorization extent - Freshly cultured inoculum (5%, v/v) was inoculated into 50 ml conical flask of dye containing sterile nutrient broth. The experiment was conducted at 37 °C. This study was carried out for 96 h under static condition. 2 ml dye containing culture was collected and centrifuged at 8000 rpm for 10 min. Decolorization percentage was estimated by measuring the absorbance at 635 nm (λ_{max}) using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). Decolorization extent was measured by the following equation:

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

Here, OD1 is the initial absorbance before decolorization, ODt is the absorbance after decolorization.

2.5. Effects of different physicochemical parameters on dye decolorization – Effects of different physicochemical parameters on dye decolorization was observed and optimized. From the bacteria, one isolate from the genus *Pseudomonas* (isolate 9), which was prominent decolorizer during screening was selected. The effect of initial dye concentration was studied in nutrient broth media having 50 to 1000 mgL^{-1} of dye.

pH of the media was adjusted to 7.0 and experiment was conducted at 37 °C. Effect of incubation period on dye degradation was observed for 96 h using 200 mg L^{-1} of dye containing media at 37 °C. pH of the nutrient broth media was 7.0. To estimate the optimum temperature, the study was done at 30, 37 and 45 °C. The initial pH of the media was adjusted to7.0. The effect of initial pH on the decolorization was investigated and for this media were adjusted to pH 5.0 to 9.0. The experiment was done at 37 °C.

2.6. Statistical analysis - For statistical analysis, *t* test was used and 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. Isolation, screening and identification of reactive dye decolorizing bacterial isolates – Heterotrophic bacteria were isolated from dyeing mill effluent. From the nutrient agar plate used for bacterial culture, ten isolates were selected randomly. Investigation for dye decolorizing capability of these bacterial isolates was done. Reactive Blue dye (200 mgL^{-1}) was used for screening of dye decolorization of these 10 isolates and was carried out in dye containing nutrient broth media for seven days (**Table 1**).

Six isolates showed positive result in the decolorization of dye and were chosen for further study. Decolorization percentage and number of dye decolorizing bacteria increased with the extending incubation period. Decolorizing isolates were identified on the basis of cultural, morphological and biochemical characteristics. These characteristics indicated two bacterial isolates as *Pseudomonas*, two as *Bacillus*, one as *Alcaligenes* and one as *Aeromonas*. The characteristics used for dye degrading bacterial identification are summarized in **Table 2**. *Pseudomonas* sp. (isolate 9) was chosen for future study as it was a prominent dye decolorizer during screening.

Table 1: 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
Reactive Dark Blue	24 h	-	-	-	-	-	-	-	+	-	-
	48 h	-	-	-	+	-	-	-	+	+	-
	72 h	-	-	-	+	-	-	-	+	+	+
	96 h	-	-	-	+	-	-	-	+	+	+
	120 h	-	-	-	+	+	-	+	+	+	+
	144 h	-	-	-	+	+	-	+	+	+	+
	170 h	-	-	-	+	+	-	+	+	+	+

Table 2: Morphological and biochemical characteristics of bacterial isolate

Morphological and biochemical tests	Bacterial isolate 9
Shape	Rod
Motility	+
Catalase production	+
Oxidase production	+
Gram staining	-
Indole test	-
Methyl red test	+
Voges-Proskauer test	-
Triple Sugar Iron Agar test (Slant/Batt)	K/A
Citrate utilization	+
Maltose fermentation	-
Lactose fermentation	+
Glucose fermentation	-
Spore test	-
Bacterial genus	*Pseudomonas* sp.

Here, K/A = Red/Yellow

3.2. Effects of incubation period on dye decolorization - We studied the effects of incubation period on decolorization of Reactive Blue dye by this bacterial isolate. It was observed by measuring dye decolorization extent at different intervals of time at 37°C and pH 7.0 (**Fig 1**). The results recorded for *Pseudomonas* showed that there was a sharp increase in dye decolorization at 24 hours and the decolorization percentage was more than 67 % of the entire decolorization percentage. After 24 hours with the extended incubation period, the progress in color removal grew slightly. The difference between the result recorded at 72 hours and 96 hours was only 2 % color removal. In literature, *Pseudomonas aeruginosa* decolorized Orange 3R dye by 93.06% (Jadhav *et al*., 2011).

3.3. Effects of initial dye concentration on dye decolorization - Initial dye concentration is an important parameter in decolorization study because it has powerful inhibitory effects on dye decolorization (Kalme *et al*., 2007). The dye decolorization extent reduced with increase of initial concentration of dye. Decolorization activity of *Pseudomonas* sp. was studied using various concentrations of Reactive Blue dye ranging from 50 to 1000 mg L^{-1} (**Fig 2**). From the result it can be concluded that with extended initial dye concentration, the decolorization percentage decreased. Highest decolorization obtained by *Pseudomonas* sp. used in this study was approximately 82% with 200 mg L^{-1} of Reactive Blue dye. In previous literature for the decolorization of azo direct blue 151 and azo direct red 31, *Bacillus* species showed maximum decolorization (95-97%) for 200 mg L^{-1} of dye concentration (Lalnunhlimi and Krishnaswamy, 2016). At 500mg L^{-1} of the dye concentration decolorization decreased drastically. Decolorization was completely inhibited when 1000 mg L^{-1} of dye was used.

3.4. Effects of temperature on dye decolorization – Decolorization extends of *Pseudomonas* sp. was observed using different temperature ranging from 30 to 45 °C. The temperature recorded for highest dye decolorization by *Pseudomonas* sp. was 37 °C. More-

over, a significant and appreciable decolorization was obtained at 30 °C (**Fig 3**). The optimum temperature for maximum Reactive Blue dye decolorization by this isolate was in harmony with the decolorization by *Pseudomonas* sp. for malachite green, fast green, methylene blue and congo red (Mali *et al*., 2000) and decolorization of fast red by *B. subtilis* (Mona and Yusef, 2008; Uddin *et al*., 2017). Decolorization activity was strongly inhibited at 45°C. This might be due to loss of cell viability or enzymes deactivation used for decolorization (Çetin and Dönmez, 2006). From the study, it can be concluded that for dye decolorization by this isolate, 30-37°C might be the best temperature.

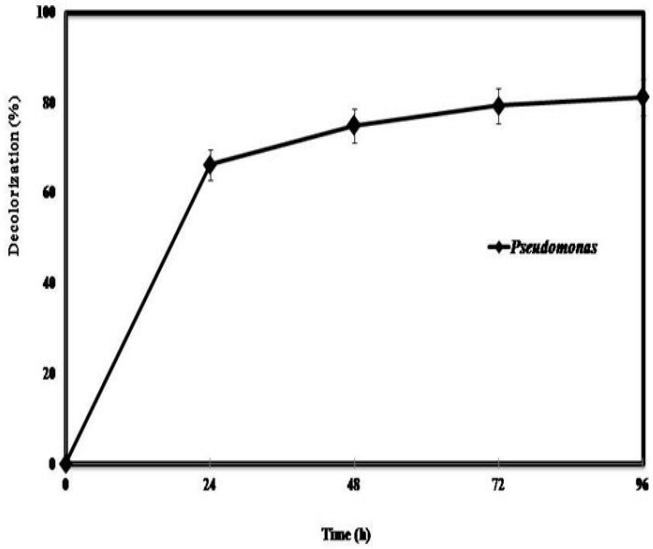

Fig 1: Effects of incubation period on dye decolorization. The concentration of the dye was 200 mg L^{-1}. The decolorization extent was measured for 96 h of cultivation at 37° C with the initial pH 7.0 and 5% inoculum.

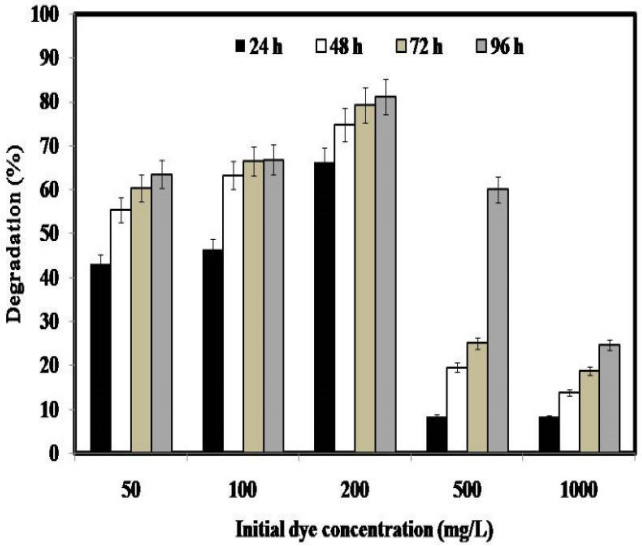

Fig 2: Effects of initial dye concentrations on dye decolorization after 96 h of incubation with 5% inoculums. The initial pH in and the temperature were 7.0 and 37 °C, respectively.

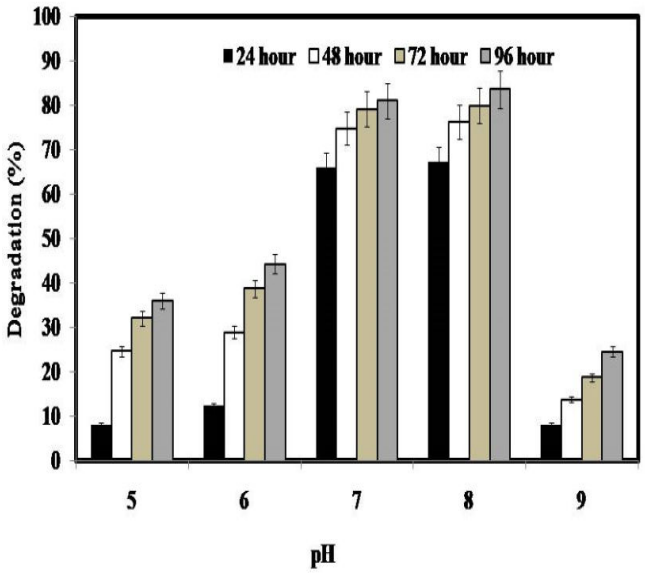

Fig 3: Effects of temperature on dye decolorization after 96 h of incubation with 5% inoculum. The initial dye concentration and initial pH were adjusted to 200 mgL^{-1} and 7.0, respectively.

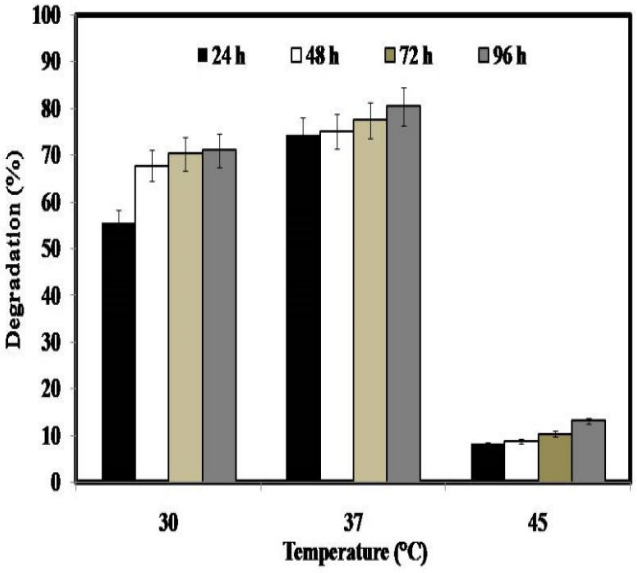

Fig 4: Effects of pH on dye decolorization after 96 h of incubation with 5% inoculum. The initial dye concentration and the temperature were 200 mgL^{-1} and 37 °C, respectively.

3.5. Effects of pH on dye decolorization - We optimized the decolorization pH as it is an important environmental parameter affecting the decolorization of dye by bacteria. The effect of initial pH across a range of pH values from 5.0 to 9.0 on decolorization by *Pseudomonas* sp. was investigated using Reactive Blue dye. The maximum dye decolorization by this isolate was obtained at pH 8.0 (**Fig 4**). However, significant level of dye decolorization (82%) was also supported by pH 7.0. Moreover, better dye decolorizations by this bacterial isolate were obtained at pH 5.0-9.0. This finding indicated that this isolate could decolorize reactive dye within a wide range of pH, indicating that this strain is a potential organism for practical bioprocessing application. The optimum pH observed for dye decolorization by *Pseudomonas* sp. in present study is comparable to the decolorization of *Bacillus* spp., *Enterobacter* sp. and *A. faecalis* PMS-1 (Shah *et al*., 2012; Lalnunhlimi and Krishnaswamy, 2016). Ability of bacterial isolates to pH tolerance is very significant as under alkaline condition binding of reactive azo dyes with jute and cotton fibre occur through addition or substitution process (Wang *et al*., 2013).

4. CONCLUSION:

Reactive dye degrading bacteria were isolated from effluent of dyeing industry and identified based on morphological and biochemical characteristics. Biodegradation depends on various physico-chemical parameters. *Pseudomonas* sp. showed significant dye decolorizing activity and this could tolerate up to 1000 mg L^{-1} of Reactive Blue dye. For high degradative and decolorizing ability against reactive dye, it can be predicted that *Pseudomonas* sp. has a suitable application potential for biotransformation of dyeing industry 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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