



## Bioremediation of Textile Dyes Wastewater: Potential of Bacterial Isolates from a Mining Soils and Wetlands

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### ABSTRACT

*New bacteria that was provided from contaminated soils surrounding the coal, aluminum, salt mines and wetland separated, synthesized and characterized. The achievements show that this soils and waters have five disposed bacteria consist of Microbacterium SP, Micrococcaceae Bacterium, Planomicrobium SP, Brevndimonas Aurantiaca and Halomonas SP. Secondly, the result of the potential of removal activity study of new bacteria, with respect to basic blue 41 and disperse red 177 dyes are presented. So, in the batch system the influence of pH, bacteria dosage, temperature, dyes initial concentration and time was investigated. Finally, the study of removal mechanism show that the biodegradation is the governing mechanism. So, we can confirmed that these natural and locally available bacteria showed a great efficiency for the removal of dyes from the aqueous solution without any unsafe by-product, also can be utilized for other water pollutants. Prog. Color Colorants Coat. 12 (2019), 155-161© Institute for Color Science and Technology.*

### 1. Introduction

Contamination of water by dyes is a global problem [1, 2] accordingly nowadays everybody knows that all type of dyes lead to many problems for human and water environment [3]. In the environment the main sources of dyes is anthropogenic contamination, including textile industrial wastes and other output of industrial activities and factories [4]. Several conventional methodologies such as biological [5, 6], chemical [7, 8] and physical [9, 10] are available for dyes and other pollutants removal from wastewaters. Generally, these methods are expensive or ineffective sometimes, especially when the dye concentration is high [11]. Among all the treatments technique that proposed, biological removal using bacteria is one of the most popular and costless method. It is now recognized as an effective, efficient and economic

method for water decontamination applications and for separation to pilot purpose [7]. Nowadays, the remediation of pollutants by natural living organisms has been widely reported such as bacteria [12], fungi [13], enzymes [14] and biological adsorption [15]. These living beings have natural base and they are environmental friendly and it is possible to be growth most of them or be applied in different environments, but low researches has focused on dye removal by natural bacteria. Bacteria that live in contaminated soils and wetlands are resistant to pollutants and they have high potential for the removal of contaminants, probably.

So, the objective of the present study is focused on the development of soil and wetlands in situ bacteria for removal of two dyes consists of basic blue 41 (BB-41) and disperse red 177 (DR-177) that because of their

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environmental significance were selected. BB-41 dye is an applicable dye for textile industries but its existence at water resource in higher than standard concentration lead to many problems that was reported by researchers already [16]. DR-177 dye is another dye found in wastewaters that its concentration in the discharging water must be less than the standard values to prevent the death of living organism [17]. The use of bacteria for the removal of organic dyes particularly BB-41 and DR-177 from water have been the subject of numerous publications in recent years [12, 18, 19]. In view of that potentially low cost process for the remediation of wastewater that contaminated by organic dyes have been discussed. The present study is focused on the development and preparation of bacteria for removal of BB-41 and DR-177 dyes. So, in the batch system the effects of pH, bacteria dosage, pollutant initial concentration, remediation time and temperature on dye removal capacity were investigated.

## 2. Materials and Methods

### 2.1. Materials, samples collection and bacterial isolation

All chemicals consist of Sodium Hydroxide, Chloridric Acid, BB-41 and DR-177 was purchased from Merck Company. The bacterial isolates were isolated and identified from contaminated soil samples at Jajarm aluminum mine (37° 03' 18.2" N, 56° 28' 52.5" E) and water of Mareh wetland (34° 57' 46.0" N, 51° 18' 19.6" E) from Semnan, North Khorasan and Qom provinces of Iran. For isolating bacterial, one gram of soil or one mL of water samples were used to prepare the serial dilution (0.1, 0.01, 0.001 and 0.0001) in double sterile water and 200  $\mu$ L of each dilution solution was cultured in LB agar medium (Merck KGaA). The solid medium plates were incubated in condition with 28 $\pm$ 2 °C for 72 h. According to the morphology, different bacterial colonies were selected and sub-cultured in solid LB agar medium at 28 $\pm$ 2 °C for 48 h and then the pure colonies were used to molecular identification and determination of dyes remediation.

### 2.2. Molecular identification of isolated-bacterial

The genomic DNA of bacterial colonies was extracted using heat and cold method in five cycles (90 and 20°C for 3 min). The quality and quantity DNA were checked by Nano Photometer (Implen N50). The

universal primers (F4: 5'- CCGCCTGGGGAGTACG-3' and Rn2: 5'- GACGGGCGGTGTGTAC-3') were used to amplify partial sequence of 16S rRNA region of isolated bacterial. The sequence of amplified DNA fragments was determined by MacroGen Inc., Seoul, South Korea. Then, the BLASTn tool of NCBI databank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was applied to find the related bacterial to our query (DNA sequences).

### 2.3. Wastewater remediation by bacteria

Stock solutions of 1000 mg/L were prepared by dissolving appropriate quantities of BB-41 and DR-177 dyes, respectively in a liter of double distilled water. Working solutions were prepared by diluting each stock solution to give the desired concentrations and followed by batch remediation studies at ambient temperature on a multi stirrer hot plate to investigate the decolorization processes. Known dosage of bacteria was added separately to a 100 mL of the each metal ions solution, thoroughly mixed, and allowing sufficient time for equilibrium. Fast filtration followed and remaining dye concentration were determined directly in the supernatant solution by spectrophotometer Unico-2300 model. The percentage removal of dyes from aqueous solution was computed by equation 1.

$$\text{Removal (\%)} = ((C_0 - C_t) / C_0) \times 100 \quad (1)$$

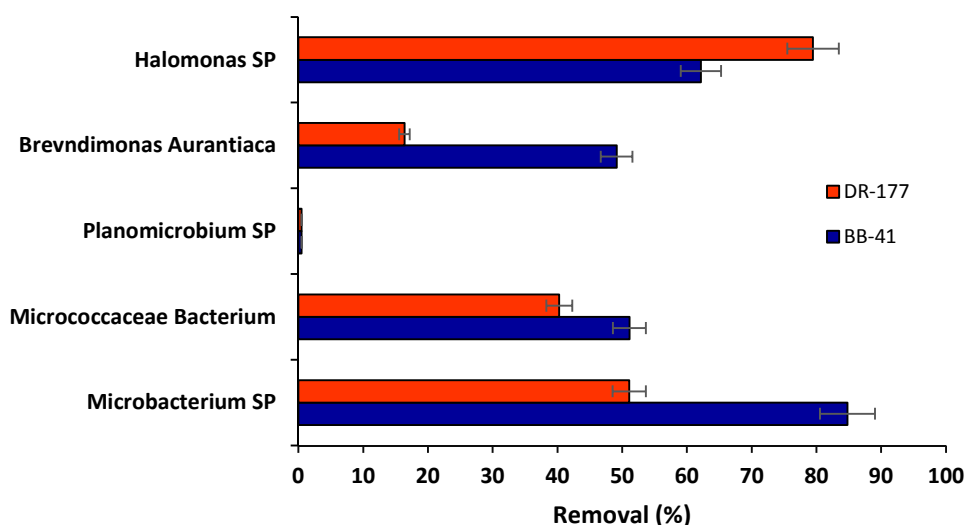
Where,  $C_0$  and  $C_t$  are the initial concentration and concentration at time  $t$ , respectively.

## 3. Results and Discussion

### 3.1. Suitable bacteria selection

In this collected soil samples, five bacteria consist of *Microbacterium SP*, *Micrococcaceae Bacterium*, *Planomicrobium SP*, *Brevndimonas Aurantiaca* and *Halomonas SP* detected. In the first step this bacteria applied for decolorization of BB-41 and DR-177 dyes and the results show that in Figure 1.

According to Figure 1, *Planomicrobium SP* bacteria don't have any removal for both dyes. Also, *Microbacterium SP* and *Halomonas SP* bacteria have higher efficiency (more than 80%) more than others for BB-41 and DR-177 dyes, respectively. So, *Microbacterium SP* and *Halomonas SP* bacteria were selected for BB-41 and DR-177 dyes removal and the optimization of operational parameters.



**Figure 1:** Remediation of BB-41 and DR-177 dyes by different bacteria (Initial concentration: 200 mg/L, Optical density: 2, Temperature: 30 °C, Time: 72 h and natural pH).

### 3.2. Effect of pH

The pH of the solution is an important factor that controls the decolorization process. The effect of pH on the dye removal by *Microbacterium SP* and *Halomonas SP* bacteria was investigated for different pH values. Here say the note is necessary that BB-41 and DR-177 dyes are soluble in all range of pH values and don't have any limitation for pH consideration. According to Figure 2, BB-41 and DR-177 dyes removal is a function of pH value.

As it is shown in Figure 2, firstly dye removal was increased with increasing at pH value and then the decolorization will fixed and don't increased. For *Microbacterium SP* and *Halomonas SP* bacteria the maximum removal of BB-41 and DR-177 dyes take place at 6 and 5 pH values, respectively.

### 3.3. Effect of dyes initial concentration

The effect of BB-41 and DR-177 dyes initial concentration on the decolorization process by *Microbacterium SP* and *Halomonas SP* bacteria was investigated by changing at the dyes concentration for 100, 200, 300, 400, 500 mg/L in optimal pH according to section 2.5 (Figure 3).

The remediation efficiency of BB-41 and DR-177

dyes was increased by increasing the initial dyes concentration for concentration less than 300 mg/L and for concentration more than 300 mg/L bacteria is poisoned due to high concentrations of contaminants.

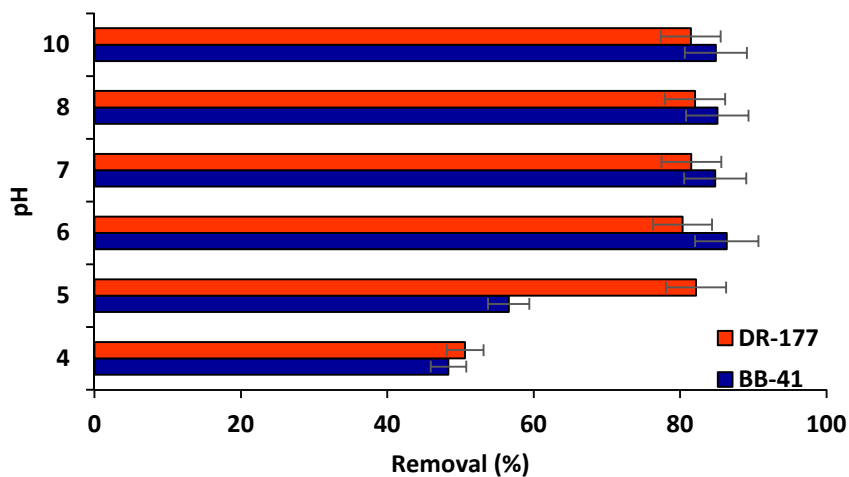
### 3.4. Effect of remediation time

The effect of BB-41 and DR-177 dyes remediation time on the decolorization process by *Microbacterium SP* and *Halomonas SP* bacteria was investigated by changing at the time for 24, 36, 48, 60, 72 and 96 h in optimal pH and initial concentration 300 mg/L (Figure 4).

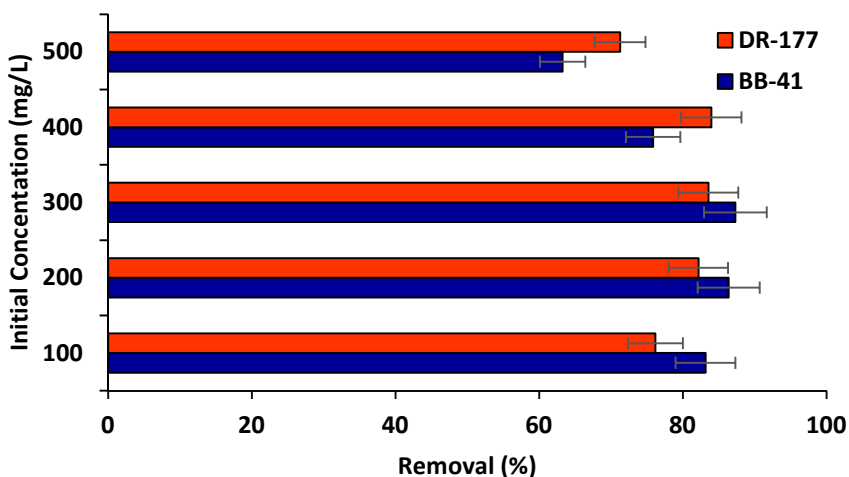
According to the Figure 4, best time for remediation of BB-41 and DR-177 dyes by *Microbacterium SP* and *Halomonas SP* bacteria is 48 h. After remediation time 48 h, the changes in the dye remediation efficiency is negligible.

### 3.5. Effect of bacteria dosage

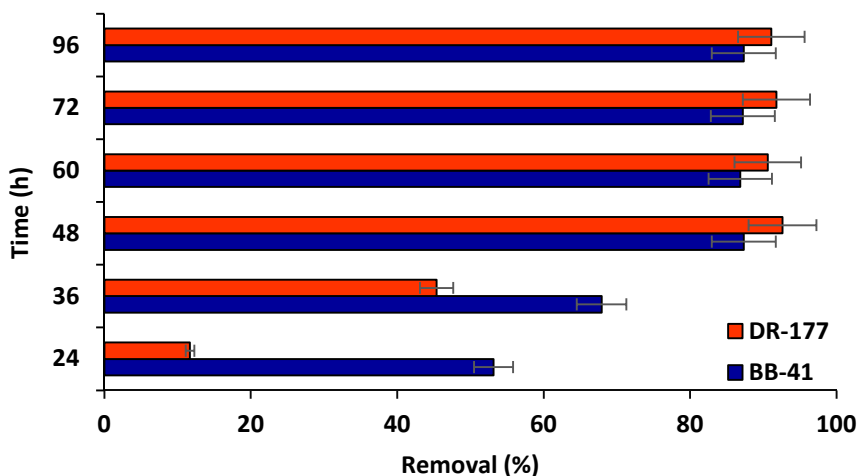
The effect of the *Microbacterium SP* and *Halomonas SP* bacteria dosage on the removal of BB-41 and DR-177 dyes which was based on the contact time 48 h was studied by changing the bacteria dosage. The results of bacteria dosage changes on the remediation of BB-41 and DR-177 dyes is showed in Figure 5.



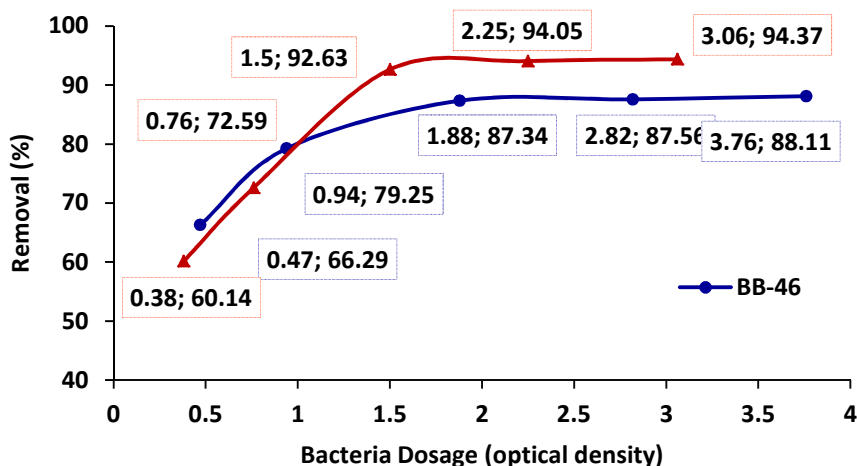
**Figure 2:** Effect of pH change on the removal of BB-41 and DR-177 dyes by *Microbacterium SP* and *Halomonas SP* bacteria (Initial concentration: 200 mg/L, Optical density: 2, Temperature: 30 °C and Time: 72 h).



**Figure 3:** Effect of dyes initial concentration change on the removal efficiency by *Microbacterium SP* and *Halomonas SP* bacteria (pH: 6 and 5, respectively, Optical density: 2, Temperature: 30 °C and Time: 72 h).



**Figure 4:** Effect of time change on the remediation efficiency by *Microbacterium SP* and *Halomonas SP* bacteria (pH: 6 and 5, respectively, Initial concentration: 300 mg/L, Temperature: 30 °C and Optical density: 2).



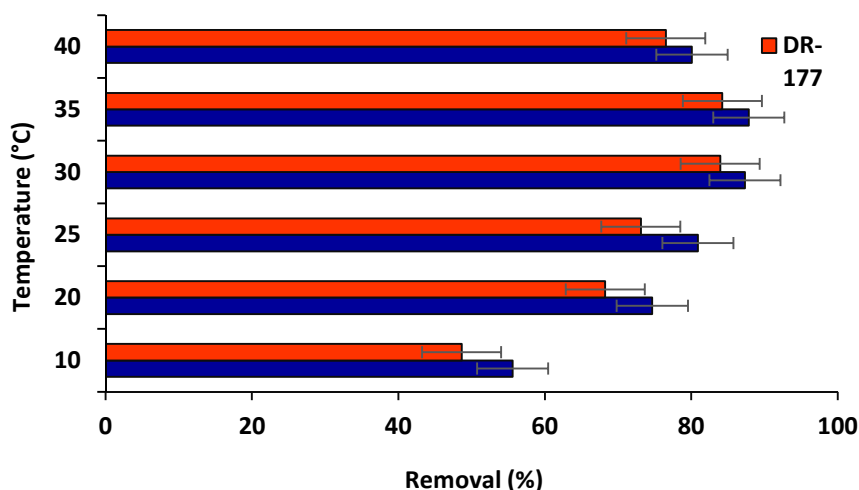
**Figure 5:** Effect of bacteria dosage change on the removal of BB-41 and DR-177 dyes by *Microbacterium SP* and *Halomonas SP* bacteria (pH: 6 and 5, respectively, Initial dye concentration: 300 mg/L, Temperature: 30 °C and Time: 48 h).

Base on Figure 5, the dye removal efficiency increase with increasing the bacteria dosage and attained a maximum value at 1.88 and 1.5 optical density for BB-41 and DR-177 dyes, respectively.

### 3.6. Effect of temperature

The effect of temperature on the BB-41 and DR-177 dyes remediation from aqueous solution was considered for six temperatures 10, 20, 25, 30, 35 and 40 °C. The results of temperature changes was showed in Figure 6.

Based on Figure 6, it is obvious that by increasing in temperature the remediation of BB-41 and DR-177 dyes increased. But, in the 35 °C temperatures the maximum activity of bacteria is observable. This phenomenon is natural, because these bacteria have maximum activity in the temperature is close to 35 °C and the literature confirmed this outcome [20]. A temperature of over 40 °C leads to the death of bacteria and thus decrease the remediation efficiency of two dyes. Colour pollution in aquatic environments is an escalating problem, despite the fact that there has been



**Figure 6:** Effect of temperature on the removal of BB=41 and DR-177 dyes by *Microbacterium SP* and *Halomonas SP* bacteria (pH: 6 and 5, respectively, Initial concentration: 300 mg/L, Time: 48 h and Optical density: 1.88 and 1.5, respectively).

substantial research into the modification of the dyeing process to improve the level of affinity/fixation of the dyestuffs onto the substrate. The recalcitrant nature of modern synthetic dyes has led to the imposition of strict environmental regulations. The need for a cost-effective process to remove the colour from wastewater produced by the textile industry has been recognised [21, 22]. The biotechnology approach to colour removal from textile effluent. Several strategies have been investigated. However, this research presented here concerns the use of in situ bacteria for the reduction of water-soluble dyes present in textile dyeing wastewater and the results is acceptable with comparison of other researches [23-25].

#### 4. Conclusions

The bacterial isolates were isolated and identified from contaminated soil samples at coal, aluminum and salt mine and water of wetland from Semnan, North Khorasan and Qom provinces of Iran. Thus, five bacteria consist of *Microbacterium SP*, *Micrococcaceae Bacterium*, *Planomicrobium SP*, *Brevndimonas Aurantiaca* and *Halomonas SP* detected. Obtained results indicated that *Microbacterium SP* and

*Halomonas SP* bacteria isolates collected in the mining soils and wetlands are effective on the bioremediation of environments and effluents of textile dyes contaminated with BB-41 and DR-177. The results show that *Microbacterium SP* and *Halomonas SP* bacteria have higher efficiency more than others for BB-41 and DR-177 dyes, respectively. Optimal condition for operational parameter consist of pH (BB-41: 6 and DR-177: 5), initial dye concentration (BB-41 and DR-177: 300 mg/L), time (BB-41 and DR-177: 48 h), bacteria dosage (BB-41: 1.88 and DR-177: 1.5 optical density) and temperature (BB-41: 35 and DR-177: 35 °C) were determined for removal of BB-41 and DR-177 dyes, respectively and the removal efficiency is more than 90 %. For concentrations less than 100 mg/L, the amount of pollutant removal is 100%. Due to do not produce dangerous hazardous by-products, these live organisms are very practical and inexpensive and can be used for removal of toxic elements, oily molecules and other organic pollutants.

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