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INVESTIGATION OF BRILLIANT BLUE R DYE BIOREMOVAL CAPACITY OF *Trichoderma sp.*

ABSTRACT

In the current study bioremoval of the reactive dyes; Brilliant Blue R, Remazol Brilliant Blue R, Reactive Red 120, Reactive Black 5 and Reactive Orange 14 by *Trichoderma sp.* were investigated. The bioremoval yields were 90.63%, 97.35%, 73.56%, 99.82% and 88.0% for Reactive Black 5, Reactive Red 120, Reactive Orange 14, Brilliant Blue R and Remazol Brilliant Blue R, respectively in the presence of 300mg/L initial dye concentration. The highest bioremoval yield was observed at Brilliant Blue R among the tested dyes. Therefore, further optimizations such as pH, initial dye concentration, incubation period, inoculum amount and maximum specific dye uptake were carried out with Brilliant Blue R. In bio removal experiments, sugar beet molasses which is cheap and abundant feedstock was used as a carbon source. In the presence of molasses medium *Trichoderma sp.* showed 99.24% removal yield in the presence of 315.23 mg/L Brilliant Blue R.

Keywords: Fungi, *Trichoderma sp.*, Bioremoval, Brilliant Blue R., Molasses

1. INTRODUCTION

Industrial developments, which are accelerated in recent years, affected the environment negatively. Dyes are commonly used products by different industries such as textiles, cosmetics, tannery, pharmaceutical, paper, plastics, electroplating or chemical. Excess usage of these dyes have polluted the environment, especially the water sources that have been affected by pollution. Because of the pollution, some serious health problems such as cancer or systematically dysfunctions increase. Besides these negative effects, textile dyes also threats the plant and animal life and it is also difficult to treat these dyes from wastewaters by chemical and physical methods because of their disadvantageous features such as overall cost or generation of secondary pollutants [1]. Therefore, there is an urgent need to develop new and effective removal methods. Among the alternatives, bioremoval of the textile dyes by microorganisms is a very cheap, effective and eco-friendly option. Microorganisms such as bacteria, yeast or fungi can remove these dyes from the environment very effectively. Thus, bioremoval of the dyes is a promising method and it has a huge potential for dye treatment [2 and 3]. Bioaccumulation and biosorption are the two important methods which are used for dye removal frequently. Biosorption is the removal

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process of toxic substances or dye polluted wastewaters with dead biological materials, while bioaccumulation is described as intracellular pollutant accumulation by living cells. These methods are rapid, cost effective and they can be performed at mild conditions [4]. Fungi are well-known microorganisms which are able to remove toxic substances such as dyes or heavy metals from the environment. They can secrete different extracellular proteins or metabolites and they also easily adapt to various environments. These unique features make fungi excellent bioagents for bioremoval [5]. For these reasons mentioned above, there are reports about dye removal from aqueous solutions using by different fungi species in the literature [6 and 7].

2. RESEARCH SIGNIFICANCE

Bioremoval can be defined as eliminating of the different pollutants by metabolic pathways in actively growing organisms [8]. Fungi have been exploited by many different areas such as dye treatment, pharmaceutical, food or enzyme production because of their fast-growing characteristics and the ability to grow in cheap fermentation media. Fungi are also suitable microorganisms for dye bioremoval [9]. By these reasons, we evaluated the dye removal capacity of the *Trichoderma sp.* fungus in the presence of different conditions. Reactive Red 120 (RR 120), Reactive Black 5 (RB 5), Brilliant Blue R (BBR), Remazol Brilliant Blue (RBB), Reactive Orange 14 (RO 14) dyes used in bioremoval studies. Important parameters such as the effect of increasing dye concentrations, the effect of the increasing amount of fungus inoculum and initial pH on bioremoval were optimized. To the best of our knowledge, this is the first report about bioremoval of Brilliant Blue R using by *Trichoderma sp.*

3. EXPERIMENTAL METHOD-PROCESS

3.1. Microorganism and Media

Trichoderma sp. was obtained from Ankara University Culture Collection. Until experiments, the fungus was kept at +4°C. Before bioremoval experiments, *Trichoderma sp.* was cultivated in 8% molasses medium which supplemented with 1g/L $(NH_4)_2SO_4$ and 0.5g/L KH_2PO_4. pH was adjusted to 5. Molasses medium was autoclaved prior to fermentation.

3.2. Preparation of the Stock Dye Solutions

Reactive Black 5 (CAS Number:17095-24-8/Sigma), Reactive Red 120 (CAS Number:61951-82-4/Sigma), Brilliant Blue R (CAS Number:610459-2/Sigma), Reactive Orange 14 (CAS Number:12225-86-4/Sigma) and Remazol Brillant Blue R (CAS Number:2580-78-1/Sigma) dyes were used. Stock solutions of dyes were prepared as 20g/L and were dissolved in the distilled water. The maximum absorbance values of the dyes were as follows: Reactive Black 5: 600 nm., Reactive Red 120: 520 nm., Reactive Orange 14: 435nm., Brilliant Blue R: 560nm., Remazol Brilliant Blue R: 590nm.

3.3. Selection of Dye Type and Effect of Initial pH on Bioremoval

In the first set of experiments, approximately 100mg/L and 300mg/L Reactive Black 5, Reactive Red 120, Reactive Orange 14, Brilliant Blue R and Remazol Brilliant Blue R dyes were added to 250 mL conical flask with 100 mL molasses. The molasses medium was allowed to incubate in a 100 rpm in shaking incubator. At the end of the incubation period, 3 mL samples were taken and centrifuged at 5000rpm for 5 minutes. Dye removal was determined spectrophotometrically. In the second set of experiments, the pH of the medium containing 100mg/L

of each dye was adjusted to 4, 5, 6 and 7. After that step, the development and dye removal capacity of the *Trichoderma sp.* was investigated.

3.4. Effect of Initial Dye Concentration on Bioremoval

BBR dye, which was removed by *Trichoderma sp.* at the highest bioremoval yield, were added to the molasses medium with increasing concentration, dye concentrations were set at 293.33 mg/L, 536.19mg/L, 906.66 mg/L, and 1161.9 mg/L. *Trichoderma sp.* was incubated at 30°C for four days at 100 rpm. At the end of the second day, 3 mL samples were taken and centrifuged at 5000 rpm for 5 minutes.

3.5. Effect of Inoculum Amount on Bioremoval

After the determination of the effect of initial dye concentrations, the amount of inoculum was doubled in order to investigate the effect of increased fungus volume. RBBR was added to the molasses at approximately 300 mg/L, 600 mg/L, 900 mg/L, 1200 mg/L. *Trichoderma sp.* were inoculated into the media and allowed to incubate for 4 days.

4. FINDINGS AND DISCUSSIONS

4.1. Effect of Dye Type on Bioremoval

Approximately 100mg/L and 300mg/L Reactive Black 5, Reactive Red 120, Reactive Orange 14, Brilliant Blue R and Remazol Brilliant Blue R were added to the growth medium in order to determine the bioremoval yield. On the 4th day of the incubation, the bioremoval yields were shown in Table 1.

Table 1. Bioremoval yields of *Trichoderma sp.* in the presence of different dyes (T:30°C, agitation speed 100 rpm, pH:5)

Dye	Initial dye concentration C_0 (mg/L)	Bioremoval (%)	Initial dye concentration C_0 (mg/L)	Bioremoval (%)
RB5	116.32	98.85±0.80	304.34	90.63±5.80
RR120	110.00	98.20±2.40	283.18	97.35±2.80
RO14	95.04	81.00±4.90	286.40	73.56±2.10
BBR	92.63	99.21±0.10	277.46	99.82±0.00
RBBR	95.27	99.73±0.20	303.63	88.00±1.40

As shown in Table 1, the highest bioremoval was observed on the fourth day of incubation with BBR dye as 99.82%. On the other hand, the lowest bioremoval was found as 73.56% in the presence of RO14. It was also observed that increasing dye concentration caused lower bioremoval yields at RB5, RO14 and RBBR dyes. Furthermore, there was no significant difference at the bioremoval yield of the BBR with the increased dye concentrations. Therefore, it can be concluded that the low toxicity of the BBR may cause higher bioremoval yields in comparison with RB5, RR120, RO14 and RBBR dyes. In a similar report, Kiliç et al., [10] investigated the bioremoval of some reactive dyes by *Lemna minor* (L.). The highest growth and bioremoval yield were detected in the presence of BBR as 59.6%. In the literature, there are some studies about the dye bioremoval capacity of the different fungi. One of them, it was found that the remazol blue bioremoval capacity of the *Rhizopus arrhizus* approximately 100% [11]. Moreover, Saravanakumar et al., [12] showed that *Trichoderma sp.* removed 88.89% of the malachite green dye at the end of the 7 days of the incubation period. Our results showed that *Trichoderma sp.* is able to remove reactive dyes.

4.2. Effect of pH on Dye Bioremoval

Trichoderma sp. showed the highest bioremoval capacity on BBR (Table 1). Therefore, further studies were carried out with BBR. In order to investigate the effect of initial pH on the bioremoval, the pH of the molasses media was adjusted to 4, 5, 6 and 7 respectively. Bioremoval yields of *Trichoderma sp.* at different pH values were presented in Figure 1.

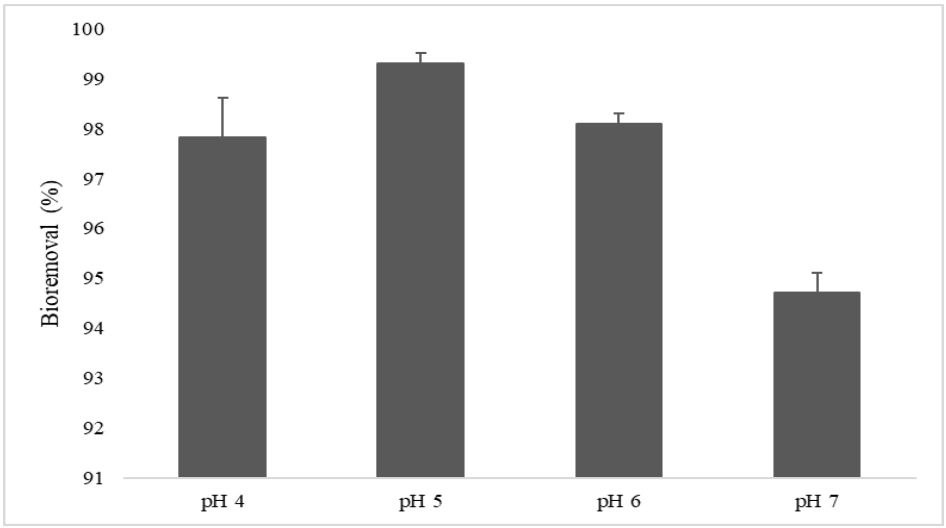

Figure 1. Bioremoval yields of *Trichoderma sp.* at different pH values

The data in Figure 1 depicts when the initial pH of the media was arranged as 4, 5, 6, 7. Bioremoval yields were detected as 97.82%, 99.31%, 98.1%, 94.7% respectively. According to the results, further studies were continued with pH 5 because *Trichoderma sp.* showed the highest bioremoval capacity at pH 5. There are similar reports on the literature. For example, Ibrahim et al., [13] found the maximum reactive red 120 bioremoval capacity of *Aspergillus* sp. and *Pleurotus* sp. as 63% and %60 respectively at pH 5. In another study, pH 5 was found as the best option for Reactive Black and HFGR bioremoval by *Aspergillus sulphurous*. In pH 5, the highest bioremoval yield was found as 93.04% at the 10 day incubation period [14].

4.3. Effect of Increasing Dye Concentrations on Bioremoval

Table 2 indicates that increasing dye concentrations caused lower bioremoval yields. It was observed that bioremoval and adsorbing dye concentrations (C_{ad}) yields increased during the incubation period. On the fourth day of incubation, the highest removal yield of *Trichoderma sp.* was 98.61% in the presence of 293mg/L BBR and the lowest bioremoval yield detected as 30.0% due to increasing dye concentrations in the presence of 1161 mg/L BBR. On the other hand, adsorbing dye concentrations (C_{ad}) increased with increasing initial dye loading up to the 906.66 mg/L. The highest C_{ad} yield was obtained from 906.66 mg/L initial RB5 loading as 785.23 mg/L at the end of the 4th day and the lowest C_{ad} was detected as 222.38 mg/L in the presence of 293.33 mg/L initial RB5 loading at the end of the 2nd day (Table 2).

Table 2. Effect of increasing dye concentrations on bioremoval by *Trichoderma sp.* (T:30°C, agitation speed 100 rpm, pH:5, inoculum amount: single)

Initial dye concentration C_0 (mg/L)	Day 2		Day 3		Day 4	
	C_{ad}	%	C_{ad}	%	C_{ad}	%
293.33	222.38	75.81±0.2	237.85	81.08±0.8	289.28	98.61±0.3
536.19	321.9	60.03±0.2	347.38	64.78±1.6	525.95	98.09±0.8
906.66	473.8	52.25±0.3	521.9	57.56±0.4	785.23	86.6±2.5
1161.9	296.19	25.49±0.5	319.52	27.49±0.2	348.57	30.0±0.1

There are similar reports, which depicted that increasing dye concentrations caused lower bioremoval yields. For instance, in a study about the bioremoval of reactive brilliant blue, *Aspergillus fumigatus* bioremoval yield was 100%, in the presence of 70.3 mg/L initial dye loading, on the other hand, bioremoval yield decreased to 20.6% when the initial dye loading was 734.1% [15]. In another study, *Aspergillus versicolor* removed 70.27% of the remazol blue in the presence of 230.2 mg/L dye. On the other hand, the removal rate decreased to 27.15% when the initial dye loading increased to 846.8mg/L [16]. Erdem et al. [17] investigated the bioremoval yields of increased concentrations of Remazol Brilliant Blue R (50-500 mg/L) by *Trametes versicolor*. Researchers found the highest bioremoval yield in the presence of 50mg/L as 99%. On the other hand, in the presence of 500 mg/L RBBR, bioremoval yield was detected as %47.

4.4. Effect of Inoculum Amount on Bioremoval

In order to determine the effect of inoculum amount on increasing dye amounts, 4 different initial dye concentrations were tested. In this set of experiments, *Trichoderma sp.* was inoculated the molasses medium with double volume. The amount of dye removed from the second day was calculated. The effect of single and double inoculum amounts on BBR bioremoval was shown in Figure 2. It was observed that the increasing concentration of dye with increasing inoculum amount the concentration of dye removed from the environment increased from day 2. However, increasing dye concentrations resulted in lower bioremoval yields. On the 4th day of incubation, the highest removal yield was 99.24% in the presence of 315 mg/L BBR and the lowest removal yield was 31.32% in the presence of 1203 mg/L BBR which is the highest initial dye loading in this part of the study.

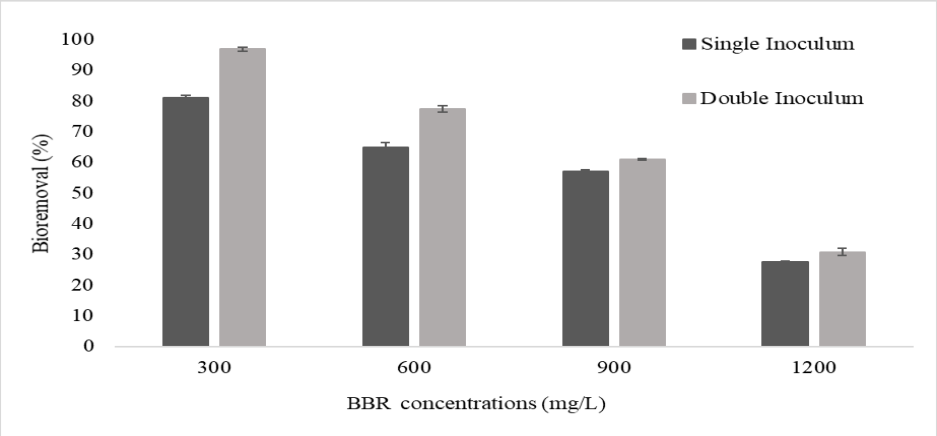

Figure 2. The effect of the increasing inoculum on the bioremoval of increasing BBR concentrations on the third day with *Trichoderma sp.* (T:30°C, agitation speed:100rpm, pH:5)

Figure 2 shows that the effect of single and double inoculum on the bioremoval of BBR with the approximate concentrations of 300, 600, 900, 1200mg/L on the 3rd day of bioremoval. In the studies conducted with single inoculum, increasing dye concentrations caused lower bioremoval yields. Bioremoval yields were 81.08%, 64.78%, 57.06%, 27.49%, in the presence of 300, 600, 900 and 1200 mg/L initial BBR concentrations respectively. Similar to single inoculum, increasing dye concentrations resulted in lower bioremoval yields in the presence of double inoculum. In the studies performed with double inoculum, it was found that 96.82%, 77.31%, 60.92%, 30.77% of the BBR were removed from 300, 600, 900 and 1200mg/L initial BBR concentrations respectively (Figure 2). Adsorbing concentrations of BBR (C_{ad}), maximum specific dye uptake (q_m), dry weights of the cells (Xm) and bioremoval yields of the *Trichoderma sp.* in the presence of single and double inoculum was given in Table 3. It can be seen that from Table 3, there was a slight increase at the C_{ad} in the double inoculum of *Trichoderma sp.* in all BBR concentrations. It was also observed that specific BBR uptake yields (q_m) did not change along with the increased inoculum amount. These results indicated that the inoculum amount did not affect the q_m. The highest C_{ad} and q_m were observed at 906.66mg/L initial BBR concentration as 794.28mg/L and 129.79mg/g respectively. Furthermore, the highest Xm and bioremoval rates were observed at the 293.33 g/L initial BBR loading as 7.11g/L and 99.24% respectively.

According to the data at Table 3, as dye concentration increases, maximum specific dye uptake increases due to microbial growth. Wang and Hu [15] reported similar observations. Researchers reported that bioremoval capacity increased to 188.1 from 34.1 mg/g when the initial Remazol Brilliant Blue R concentrations increased to 734.1 from 70.3 mg/L. In another study, 0.75 (g% w/v) Rhizopus nigrigans biomass removed about 85% of the 50 mg/L reactive green dye, on the other hand, when biomass loading increased to 1.50 (g% w/v), bioremoval yield was observed about 90% in the presence of 50 mg/L reactive green [18]. In this study, the bioremoval yield of the single inoculum was 98.61% while the bioremoval yield of the double inoculum was 99.24% in the presence of 293.33 mg/L initial RB5 loading.

Table 3. Effect of single and double inoculum of *Trichoderma sp.* on t some parameters and bioremoval yields (T:30°C, agitation speed:100 rpm, pH:5)

C_0 (mg/L)		C_{ad} (mg/L)		q_m (mg/g)		X_m (g/L)		Bioremoval (%)	
Single Inoculum	Double Inoculum	Single Inoculum	Double Inoculum	Single Inoculum	Double Inoculum	Single Inoculum	Double Inoculum	Single Inoculum	Double Inoculum
293.33	315.23	289.28	312.85	44.23±0.0	44.0±0.1	6.54±0.02	7.11±0.04	98.61±0.3	99.24±0.6
536.19	575.23	525.95	567.14	82.95±0.1	86.58±0.4	6.34±0.03	6.55±0.03	98.09±0.8	98.59±0.1
906.66	901.9	785.23	794.28	129.79±0.1	127.28±0.8	6.05±0.05	6.24±0.04	86.6±2.5	88.06±0.4
1161.9	1203.81	348.57	377.14	86.49±0.2	91.53±0.1	4.03±0.02	4.12±0.01	30.0±0.1	31.32±0.6

5. CONCLUSION AND RECOMMENDATIONS

Removal of textile dyes has gained great importance in the last decades. Biological treatment techniques become very important due to the fact that physical and chemical methods that are used in the treatment of textile wastewater are not economic, affect only certain dyes and produce intensive activated sludge. In this study, BBR dye used in the textile industry and its bioremoval on *Trichoderma sp.* were investigated. *Trichoderma sp.* effectively removed BBR in the molasses media. In the presence of high dye concentration such as 906.66 mg/L *Trichoderma sp.* adsorbed 794.28 mg/L BBR and the maximum q_m

yield was 127.28mg/g. Therefore, the usage of *Trichoderma sp.* for bioremoval of the Brilliant Blue R may quite promise.

NOTICE

This study is presented at 28-29 September 2018, Eurasian 2nd International Conference on Biological and Chemical Sciences in Ankara-Turkey has been presented as oral presentations and restructured.

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