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Decolorization of Remazol Yellow RR Gran by white rot fungus *Phanerochaete chrysosporium*

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Abstract: *In this study, the removal of color, chemical oxygen demand (COD) and aromatic group from one of the azo dyes, Remazol Yellow RR Gran, had been carried out by using one of the white rot fungi, Phanerochaete chrysosporium. Experimental studies were performed in growth media containing different amounts of dye and glucose. Color measurements were done at 436 nm wavelength using spectrophotometer, while aromatic group measurements were done at 280 nm wavelength using UV/Visible spectrophotometer. As a result of this study, the values of the removable color concentration were determined as 10 mgl^{-1} and lower. The optimum medium glucose concentration was determined to be 2 gl^{-1} during color removal processes, aromatic group measurements were done in samples in the UV region at 280 nm wavelength. As a result of the measurements, it was shown that certain amount of aromatic group remained in the model wastewater at the end of the process.*

Key words: *Decolorization, Textile dyes, White rot fungus and Phanerochaete chrysosporium*
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Introduction

Synthetic dyes are used very widely in many industries such as paper, colored photography, and textile industry. There are 10,000 types of dyestuff throughout the world and approximately 7x10^5 tones of these are produced every year. A remarkable amount of the dyestuffs is lost during dyeing processes. These losses are mainly disposed off into aquatic environment by textile and dyeing industries and some others means.

For color removal from wastewater, biological treatment systems have been widely used such as physical and chemical methods of flocculation, coagulation (Stephenson and Sheldon,1996; Kariminiaae *et al.,* 2007; Patel *et al.,* 2006; Santos *et al.,* 2007; Nisho *et al.,* 2006), adsorption (McKay *et al.*,1987; Gupta *et al.*,1990), oxidation, filtration and electrochemical methods (Lin and Peng,1996). Nowadays, the most common and standard treatments applied to textile wastewater involves biological and chemical methods (Balcioglu and Arslan,1997; Asad *et al.,* 2007; Pandey *et al.,* 2007). While various physical and chemical methods provide high color removal, they are disadvantageous, since color removal efficiency varies with dye type contained in the wastewater and these are expensive methods. Adsorption seems to be an efficient method among the color removal methods. The most commonly used adsorbent is active carbon dust. However, active carbon dust method is expensive, when the ratio of the amounts of removed color to consumed adsorbent amount is taken into consideration.

Color removal is generally realized by adsorption of the dyes using special bacteria rather than by oxidation in aerobic systems. It has been shown in the literature that some of the anaerobic microorganisms degrade dyes by reducing their nitrogen bonds, but toxic and carcinogenic compounds are often formed as a result of biological degradation (Brown and Devito,1993; Chung and Stevens,1993; Nilsson *et al.,* 2006). Nevertheless, color can be regained by contact of anaerobic degradation products with oxygen (Knapp and Newby,1995). These problems restrict the color removal in big amounts using bacteria. Because of the above-mentioned problems, faced with active sludge systems or aerobic, and anaerobic bacteria during color removal, color and organic load removal with white rot fungus was examined in this study. It has been known that white rot fungi have the ability to degrade many substances which are hard to be degraded, such as lignin, chlorinate aromatic and aliphatic hydrocarbons, and dyes, by using extracellular enzyme systems (Lin and Peng,1996; Bumpus and Aust,1985; Zaimoglu, 2006; Selvam *et al.,* 2003). The most commonly used white rot fungus species are *Phanerochaete chrysosporium, Coriolus versicolor* and *Trametes versicolor.*

In this study, color, COD and aromatic group removal using a white rot fungus *Phanerochaete chrysosporium*, in a model wastewater containing sizeable concentrations of Remazol Yellow RR gran, which has been produced by Dystar Company and is one of the widely used azo dyes, has been investigated. The biological degradation efficiencies obtained were compared with limit values in the legal regulations. During color measurements a new color parameter called Index of Transparency Parameter (DFZ = Durchsichts-farbzahl) was used in accordance with the European Norm EN ISO 7887 (Europa Norm,1994). COD values

were compared with respect to Turkish Water Pollution Control Regulation discharge limit values (Turkish Water Pollution Control Regulation,1983). As a result, in case of Remazol Yellow RR gran, which has a wide range of utilization area in textile sector and exists in aquatic environments, color and pollution load can be treated by one of the white rot fungus *Phanerochaete chrysosporium.*

Materials and Methods

Micro-organism: In the studies, a white rot fungus *Phanerochaete chrysosporium* ME 466, belonging to the Basidiomycetes group which lignolytic activity and a role in the degradation of aromatic chlorined compounds, was used. *Phanerochaete chrysosporium* ME 466 was isolated in a forest products laboratory in USA (Mileski *et al.*,1988). In order to maintain regular supply of *Phanerochaete chrysosporium* ME 466, it was cultivated on potato dextrose agar (3.9 g potato dextrose agar + 1.5 g agar /100 ml distilled water) once every 7 days and cultures obtained after 6-7 days of incubation at 37°C were kept at + 4°C for use in the future studies.

Cultivation conditions: Ten grams of Malt Extract Broth was sterilized at 115°C at 1 atm for 10 min and it was dissolved in 0.5 l of distilled water. This sterilized medium, *Phanerochaete chrysosporium* cultivated in 2 petri dishes (diameter 9 cm) for 6 days was added under sterilized conditions after being homogenized with the help of a Braun-type mixer in 100 ml sterilized malt extract broth. Then the cultures, which were grown for 3-4 days at 37°C(Lin and Peng,1996; Tatarko and Bumpus,1997), were filtered with a sterilized Millipore glass filter (diameter 0.45 µm) and the product was obtained as "wet microorganisms". This fungal mycelium was homogenized using a sterilized gas burner. Afterwards, these cultures (fungal mycelium) were transferred to batch reactors, in which color removal was realized. The concentration was kept constant at 1g culture/100 ml wastewater.

Decolorization medium: In the studies, the nutrient medium used by Fu-Ming Zhang and others (Zhang *et al.,* 1999a) was utilized as basic nutrient. According to this; varying glucose amounts (1, 2, 5 gl^{-1}), 3 mgl^{-1} $MnCl_2$, 4 mgl^{-1} $FeSO_4.7H_2O$ and 40 mgl^{-1}. $MgSO_4.7H_2O$ were used. 250 ml of phosphate buffer was added to adjust pH 5. The reason for experimenting at different concentrations of glucose was to determine the optimum glucose concentration. Nutrient medium components were added in very small amounts, from initially prepared stock solutions. Later, the above-stated amounts were used from these stock solutions. The same procedure was followed while adding the dyes. The required amount of solution was transferred from the concentrated dye stock solutions to the basic nutrient medium to obtain the desired above concentration conditions. Finally, pH values were checked. Basic nutrient media components and stock dye solution were sterilized at 121°C and 1.5 atm for 15 min, after being transferred into the aerobic reactors (250 ml Erlenmeyer flasks), each at 100 ml, and then the dye was added. Later, the obtained microorganism cultures were placed into batch reactors of 250 ml volume, in which the color removal would be realized. The wet weight concentration of the culture was adjusted to 1 g per 100 ml liquid nutritional medium. Decolorization studies were carried out at 37°C and 150 rpm for 48 hours (Zhang *et al.,* 1999b).

Table - 1: Some physical and chemical properties of Remazol Yellow RR Gran

Structure	: Granule
Color	: Deep yellow
Odor	: Odorless
Melting point	: >200°C
Density	: 0,48 g/cm^3
Aqueous solubility	: 100 gl^{-1} (20°C)
pH	: 5-6 (10 gl^{-1}, 20°C)
Flash point	: Not determined
Kindling point	: >300°C

In order to determine the biological adsorption effects of dyes on fungus, distilled water containing only the same concentration of dye and the fungus in the batch reactors was used with controls and incubation was performed under the same conditions as in the biodegradation experiments (Kapdan *et al.*, 2000).

Azo dyes: Remazol Yellow RR gran produced by Dystar company was used to examine the color removal efficiency. According to the Safety Data Sheet of Dystar, Table 1 was generated to show the general properties of the dyestuffs.

Color measurements: In the studies, standards of European Norm EN ISO 7887 were considered as basis and DFZ parameter was chosen for color measurements with a Novaspec II type spectrophotometer, which works visible light spectrum. Remazol Yellow RR gran measurements were carried out at 436 nm wavelength and the results converted to DFZ number.

Wastewater sample, after the color removal process, was centrifuged at 11000 rpm for 10 minutes, water sample treated similarly was used as control to adjust the desired wavelength. The measured absorbance values were converted to "Indexes of Transparency" (DFZ = Durchsichts-Farb-Zahl). DFZ limits determined according to European norm, are 7 m^{-1} for 436 nm, 5 m^{-1} for 525 nm and 3 m^{-1} for 620 nm (Europa Norm,1994). DFZ calculation was made according to:

DFZ= 100 ($E\lambda$ / d), where

$E\lambda$ is extinction (at a known wavelength) and

D is the thickness of sample in cm.

COD and aromatic group analysis: COD measurements were done according to the standard method (open reflux, titrimetric method) described in APHA 5220 B. Aromatic group analyses were carried out in a Jenway type 6105 UV/VIS spectrophotometer at 280 nm, after analysis of the wastewater sample for color removal, as mentioned above for color measurements (APHA *et al.*, 1999).

Results and Discussion

The most suitable carbon source that white rot fungus can utilize is glucose. Glucose concentration used in color removal

Table - 2: % Color and % total COD removal of 100 mgl^{-1} Remazol Yellow dye by using 1, 2 and 5 gl^{-1} of glucose

R. Yelow RR Gran (436 nm)	0 hr		15th hr		22nd hr		36th hr		48th hr	
	COD	DFZ	COD	DFZ	COD	DFZ	COD	DFZ	COD	DFZ
1 gl^{-1} Glucose	0	0	69	20	77	22	92	22	93	22
2 gl^{-1} Glucose	0	0	82	24	94	23	95	23	96	23
5 gl^{-1} Glucose	0	0	22	35	44	43	60	45	67	46

Table 3: Aromatic group removal (%) of Remazol Yellow dye

Time (hr)	50 mgl^{-1} R. Yellow		25 mgl^{-1} R. Yellow		10 mgl^{-1} R. Yellow	
	A280	%	A280	%	A280	%
0	1,047	0	0,641	0	0,336	0
15	0,686	34	0,427	33	0,302	10
22	0,684	35	0,42	34	0,324	4
36	0,7	33	0,42	34	0,242	28
48	0,700	33	0,43	33	0,24	29

Table - 4: Color and total COD removal (%) of Remazol Yellow dye at concentrations of 50 mgl^{-1}, 25 mgl^{-1}, 10 mgl^{-1}

R. Yelow RR Gran (436 nm)	0 hr		15th hr		22nd hr		36th hr		48th hr	
	COD	DFZ	COD	DFZ	COD	DFZ	COD	DFZ	COD	DFZ
50 mgl^{-1}	0	0	84	67	86	68	87	67	92	67
25 mgl^{-1}	0	0	79	70	89	74	91	74	98	74
10 mgl^{-1}	0	0	82	64	86	71	97	71	98	71

experiments with fungi generally ranged between 1 and 5 gl^{-1} (Zhang *et al.* 1999a; Kapdan and Kargi, 2001). During the glucose optimization in nutrient media studies, the glucose concentrations were separately kept at 1, 2 and 5 gl^{-1}. At these concentrations the color and COD removal values were investigated in the batch systems. The obtained results are given in Table 2.

In the optimization experiments, glucose amount was kept at 1, 2 and 5 gl^{-1} in the nutrient medium, and the glucose effect on color removal efficiency was analyzed. In these studies, 2 gl^{-1} of glucose was determined to have the most efficient color and COD removal capacity (Table 2). Therefore, in the rest of the studies 2 gl^{-1} glucose was used in all nutrient media.

Effect of dye concentrations on color, COD and aromatic group removal: In these experiments, the color, COD and aromatic group removal efficiencies were analyzed for dye concentrations of 50 mgl^{-1}, 25 mgl^{-1} and 10 mgl^{-1}. The results obtained are given in Fig. 1-4 and Tables 3-4.

DFZ number of 50 mgl^{-1} of Remazol Yellow RR Gran is shown in Fig. 1 as 63 m^{-1} initially. Starting with a sharp decrease during the first 15 hr, the DFZ number reaches a horizontal limit of around 21 m^{-1} at the end of the 48 hr. COD values in Fig. 4 indicate that the process starting with 2100 mgl^{-1} COD value reached at 48 hr with 171 mgl^{-1} COD value. Table 4 shows that 67% of DFZ has been removed at the end. COD removal value is, however, 84% at the end of the 15 hr, 86% at the end of the 22 hr, rises to 87% at the end of the 36 hr and stops at 92% after 48 hr. From the values in Fig. 1, the biological adsorption amount was determined to be 3%.

DFZ number of 25 mgl^{-1} R. Yellow RR Gran was 27 m^{-1} at 0 hr , as seen in Fig. 2. At the end of 48 hour, this value was 7 m^{-1}. COD concentration (see Fig. 4) decreased to 432 mgl^{-1} after 15 hr and ended as 40 mgl^{-1} after 48 hr. Percentage removal efficiency of the mentioned values was 70% for 15 hr and became stake at 74% (Table 4). COD removal value was 79%, where 89% at 22 hr and reached 98%. From the values in Fig. 2, it was determined that biological adsorption level was 8%. 25 mgl^{-1} Remazol dye showed an aromatic group removal value of 33%, as seen in Table 3.

The initial DFZ value for 10 mgl^{-1} Remazol Yellow is 14 m^{-1} as shown in Fig. 3. This value decreases down to 5 m^{-1} at the 15th hr and finally to 4 m^{-1}. Fig. 4 shows that the COD value decreases to 45 mgl^{-1} at the end of the 48 hr. Corresponding percentage values of these measurements are given in Table 4. DFZ removal increases to 64% at the end of the 15 hr and stops at 71%, while COD removal increases to 72% at the end of the 15 hr and stops at 98% for COD removal. From the values in Fig. 3, the biological adsorption amount has been determined to be 7%.

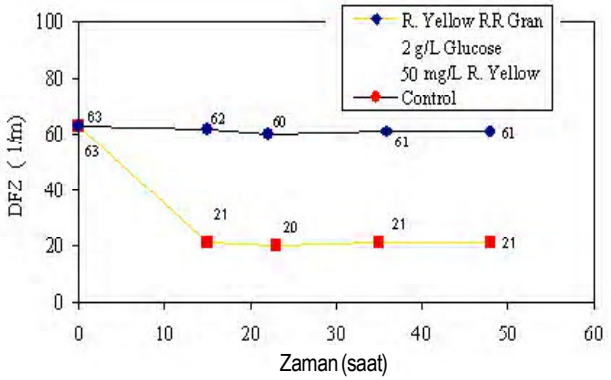

Fig. 1: Color removal of 50 mgl^{-1} R. Yellow RR Gran

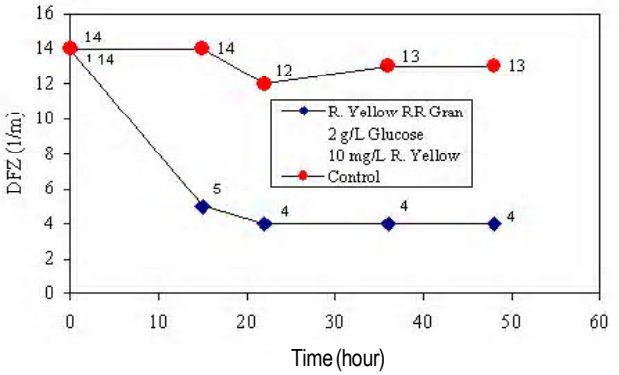

Fig. 3: Color removal of 10 mgl^{-1} R. Yellow RR Gran

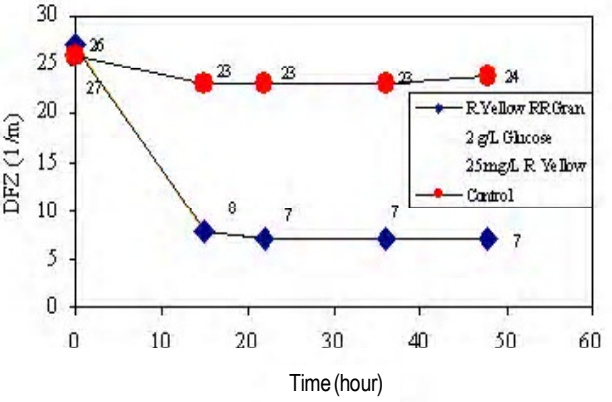

Fig. 2: Color removal of 25 mgl^{-1} R. Yellow RR Gran

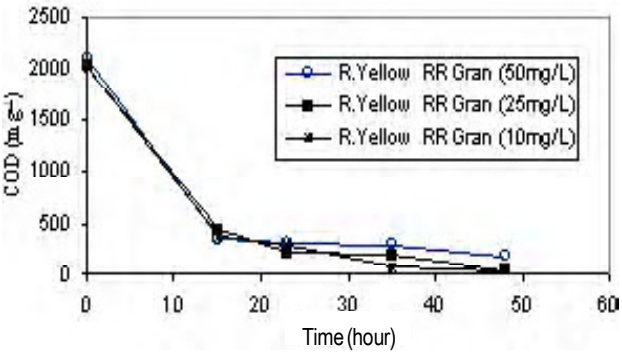

Fig. 4: Total COD removal during biological degradation of Remazol Yellow RR in concentrations of 50 mgl^{-1}, 25 mgl^{-1} and 10 mgl^{-1} (2 gl^{-1} glucose)

In this study, color, COD and aromatic group removal efficiency values of *Phanerochaete chrysosproium*, a white saprophytic fungus, were determined with a Remazol-type dye, Remazol Yellow RR Gran.

It is known that white saprophytic fungi serve as an effective means for biological degradation and as a resultant decolorization of growth medium containing a limited amount of nitrogen taken place. Therefore, glucose optimization was done by using three different growth media with 1, 2, and 5 gl^{-1} glucose (no nitrogen is present in these media) to determine the optimum media. The medium with 2 gl^{-1} glucose was selected due to both optimum. DFZ efficiency and a low amount of non-removable COD value. This result shows that glucose level is highly dependent on removable color and COD value.

The most convenient nutrient medium (containing 2 gl^{-1} glucose) was used to investigate the effect of dye concentration on color, COD and aromatic group removal efficiencies. For R. Yellow concentration of 50 mgl^{-1}, 67% of the DFZ was removed and it has been observed that the studies revealed in a high DFZ value of 21 m^{-1} after 48 hr of biological degradation. COD removal was observed to reach an efficiency of 92% and the value decreased to 171 mgl^{-1}. According to these results, since limit DFZ value is 7 m^{-1} for yellow color in European Norm EN ISO 7887, the resulting DFZ value of 21 m^{-1} as determined, appears to be far above the limit value (Fig. 1 and Table 4).

After 48 hr of biological incubation of 25 mgl^{-1} Remazol Yellow, it was observed that 74% of DFZ was removed to give a DFZ value of 7 m^{-1}. COD removal efficiency was 98% after 48 hr and the final COD concentration was 40 mgl^{-1} (Fig. 2). This is in accordance with EN ISO 7887 for R. Yellow. After 48 hr of biological degradation with 10 mgl^{-1} R. Yellow, 71% of DFZ was removed (down to 4 m^{-1}). COD removal value reached 98% after 48 hr and COD value decreased to 45 mgl^{-1}. The DFZ value of 4 m^{-1} provides the desired discharge limit, since the limit value is 7 m^{-1} in EN ISO 7887. In conclusion, the removable concentration for Remazol Yellow RR Gran has been determined to be 10 mgl^{-1} and below.

Yellow RR Gran's biological adsorption was determined to be 3 and 8% for the color removal of *Phanerochaete chrysosporium* in batch reactors.

It has been determined that hydraulic retention time should be minimum of 15 hr and maximum of 48 hr for the batch reactor in order to meet the criteria of EN ISO 7887. The fact that most of the color removal takes place in the first few hr, is also supported by other workers studies (Yesilada *et al.,* 2003).

In the batch reactor, COD values after 48 hr of hydraulic retention time meets the requirements of Turkish water pollution control regulations limit discharge values, which are in the range of 200-300 mgl^{-1} of COD. Wastewater COD is influenced by glucose, which is used while preparing the wastewater, and is a component of the nutrient media. For this reason, COD removal is carried out by the white rot fungus rapidly. This fact is supported by the literature data.

Aromatic group measurements show that some portion of the aromatic group was non-degradable in the model wastewater. This situation is supported by the results of other investigators (Heinfiling *et al.*, 1997).

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