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The Decolorization of Reactive Textile Dyes by *Pleurotus Sajor-Caju*

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Abstract

Textile industry is one of the most important industries of Turkey. The exit water of textile industry, which involves textile dyes including variety of chemicals and complicated structure, harms the environment widely. Biological treatment methods has attracted the attentions not only because of its low investment and operating costs but also because of being a simple and environment friendly treatment process. White rot fungi are used as a biological system in degradation and decolorizing of textile dyes.

In this study, the biological purification of dyes intended to lower the cost and as soon as possible. In this study, some of the reactive textile dyes (Blue 49, Orange 12, Orange 13, Red 31, Black 5, RBBR). Pleurotus sajor-caju has been optimized for parameters that affect the color removal. At different pH (3.5, 4.5, 5.5, and 6.5) dye concentration (25, 50 and 100 mg/l), shaking speed (100, 150 and 200 rpm) and temperature (25, 30, 35 and 40°C) investigated the effect of decolorization. Optimum conditions, the degradation of dyes percentages ranged from %70 and %93. The most resistant to degradation dye of the Orange 12 and Orange 13 was. Blue 49 and RBBR is the fastest degradable. pH 4.5 optimum conditions for decolorization of dye concentration of 50 mg/l, shaking speed of 200 rpm and a temperature of 30°C were determined.

Keywords: White Rot Fungi, Pleurotus sajor-caju, Decolorization and Textile dyes

INTRODUCTION

In our country because of the rapid increase in the population, unplanned urbanization, industrial areas not including additional units such as treatment plants we encounter environmental problems. These substances, increasingly released to the nature, cause serious problems on water, air and earth, which are the milestones of living creatures.

In addition to urban and sewage water, industrial effluent is the most important factor affecting the surface water sources. Organic, inorganic and variety of dye substance pollution caused by a lot of industrial effluent such as specially, cosmetics, dye, paper, leather, food, plastic, etc. threatens the human health and ecological balance. Thus, it is vitally important for such pollutant substances to have been removed from the industrial waste [1, 11].

Effluent generated from the textile industry causes serious problems [9]. Textile industry, which is one of the most important branches of industry in Turkey, has a changeable structure because of the variety of the raw material, chemical material, the operations carried out and the diversity of the technology applied for each process. This changeable structure reflects on the purification technologies which were applied to the textile effluent and because of this it has been difficult to apply a standard purification method [10].

After the first synthetic dye substance was found by W. H. Perkin in 1856, dyestuff industry was considerably developed especially in England, France, Germany and Switzerland. However, today dyestuff is produced over 10000 different kinds and approximately over 7 x 10^5 tons. Moreover, almost 10-15% dyestuff produced were discharged as waste material [3, 16].

Although colorful compounds organically constitute of the small portion of effluent in general, at the points they are discharged they cause the place be polluted aesthetically. Colorful effluent, which was discharged, reduces the transparency of light which is vital for the photosynthesis of primary producers living in water and harms the ecosystem dramatically [8]. Therefore, decolorizing of the colored effluent resulting from textile and dyestuff producing industries has been an important scientific field of interest. In addition to physical, chemical and physico-chemical decolorization studies, biological operations of color removing studies have accelerated [17, 21].

Because of the high cost of physical and chemical methods suggested for the textile industry effluent and their not being able to be used for each kind of dye, their use is limited. The studies, which have been conducted, recently emphasized the existence of many kinds of widespread microorganisms, which have the ability to remove the variety of dye effluent, and brought the biotechnological methods into the forefront. That is theoretically because of biological treatment systems producing less mud, low cost or not producing harmful side products for the consumer environments features when compared with chemical and physical purification methods, it has been accepted as an ideal solution for the effluent purification of the textile industry [20, 13].

In textile industry the dyes which have different structures, have been used in a short time and in the same institute. Therefore, effluent resulting from textile industry is very important because of its composition. It has purification out that in order to refine the effluent of textile, a pretty big unspecific process is required. This required unspecifity could be obtained by widely known biological systems such as using the lignin peroxidase taken from lignolytic fungi or the unspecific reduction processes catalyzed by variable bacteria under anaerobic conditions [23].

White rot fungi is the organism, which have been widely used in the biological refining studies being conducted in the field of waste and environment biotechnology [30]. White rot fungi which are in the category of basidiomycetes, are released depending on the intensive industrial activity. It is known that they play a role in the oxidation of the organic compounds, which have variety of molecular structures, particularly laccase and other enzymes they produce in order to eliminate the environmental pollution [15]. Laccase,

Mn-peroxidase, Lignin peroxidase and NADH peroxidase (NADH oxidase) extracellular enzymes synthesized by the white rot fungi could be given as the examples of the fungi used in the biotechnological studies such as *Trametes versicolor*, *Funalia trogii*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *P. eryngii* [29]. From the literature studies it was confirmed that white rot fungi was widely used in order to decolorization of the textile effluent in all over the world [24, 10, 17, 7, 28]. Dyestuffs, which come out of textile industry, are released around in nature. It is a vital necessity that before it was released to the nature appropriate refining methods had to be applied to the dyestuffs, which include the most important pollutant factor. In our study, the aim is to remove dyestuffs biologically with low cost and in a short time. In this respect, *Pleurotus sajor-caju* which is a white rot fungi, was used.

For this purpose, the decolorization of some reactive dyes used in the textile industry in Turkey was experimented with *P. sajor-caju* pellets in a shaking culture environment. Also, for the decolorization of the dyestuff appropriate ambient conditions were determined (pH, dye concentration, shaking speed (rpm), temperature). In addition to this, the toxicities of the samples, which were taken during the decolorization period, were determined by using the *Artemia salina* toxicity test. Thus, the probable effects of metabolites, which existed during the degradation and at the end of it, to the naturel life, were specified.

MATERIAL and METHODS

Dyes

The trade names and color index (C.I.) numbers of the reactive dyes used in the decolorization are; Cibacron Blue 3R (C.I. Reactive Blue 49), Reactive Golden Yellow HR (C.I. Reactive Orange 12), Reddish Orange (C.I. Reactive Orange 13), Reactive Red H8B (C.I. Reactive Red 31), Remazol Black (C.I. Reactive Black 5), Remazol Brilant Blue R (C.I. Reactive Blue19). 1% of the stock solutions of the dyes were prepared by using distillated water and it was sterilized at 121°C for 15 minutes. Stock dye solutions were kept in dark bottles at +4°C. Dyestuff solutions were used by adding to the medium under hygienic circumstances the amount of which was enough to get the necessary concentration after sterilizing in autoclave then cooled down until the room temperature.

The Environment of Microorganism and Culture Used in Decolorization Studies

In the studies *P. sajor-caju*, which belongs to the Basidiomycetes class, was used. During the study and after that the cultures potato dextrose agar (PDA) were kept at +4°C *P. sajor-caju* was planted in the PDA plates and kept at 30°C for 7 days of incubation. At the end of the incubation, the diameter of 1 centimeter discs including micelles and spores were taken out (10 disks) and transferred to erlenmayer flasks in which there is Kirk Medium [12]. They were incubated at 30 °C and and 100 rpm for 4 days. At the end of the incubation period, after being filtered under the sterilized conditions it was homogenized with the homogenizator (Heidolph) and the product was obtained as a wet microorganism weight. In the studies 1 gram microorganism was added as wet weight. The percentage of decolorization in the medium was expressed as (%). The formula used in the decolorization percentage is given below. While determining the optimum conditions for the decolorization, each study was carried out with two parallels.

Decolorization (%): (CO-C1)/COx100

(Co; initial colour concentrations and C1 final concentrations)

In all decolorization experiments the culture environment was inoculated with pellets, which are in exact wet weight. To determine the dry weight, which is equal to this wet weight, pellets were weighed with drying paper after they were dried from 12 hours at 80°C. By using this method, additional to the dry weight which is equal to the amount of wet inoculum at the beginning, at the end of the incubation period the amount of biomass in the dye and not dye medium.

Drawing the Standard Curves of the Dyestuffs

The dyestuff 1, 5, 10, 20, 30, 40, 50 mg/l of concentration series were prepared by being solved it in distillated water. The maximum absorbance wave length of each dyestuff was determined. To do it, visible spectrophotometer (JascoV-530 UV/VIS Spectrophotometer) was used. Spectrum scan was made between 300-700 nm. Afterward, the absorbance rate of each agar was determined by reading the values on the spectrophotometer (Blue 49 (600 nm), Orange 12 (430 nm), Orange 13 (490 nm), Red 31 (545 nm), Black 5 (600 nm), RBBR (600 nm).

Determining the Capacity of *P. sajor-caju* Decolorization Textile Dyes

P. sajor-caju decolorization skill was tested with the previous studies. The skill of textile dyes used was pH 4.5, dye concentration was 25 mg/l, shaking speed was 100 rpm and temperature was experimented as 30°C [26, 31, 6]. According to the data gained from this study, in order to increase the success of *P. sajor-caju*, which had the highest decolorization percentage in the shortest time and the best result, parameters like pH, dye concentration, shaking speed and temperature were changed and if faster and more decolorization happened or not was experimented.

Determining the Optimum Conditions for Decolorization

In this stage of the study, *P. sajor-caju* optimum conditions of the decolorization were determined. To do this pH as 3.5, 4.5, 5.5, and 6.5 dye concentrations as 25, 50, and 100 mg/l, shaking speed as 100, 150, and 200 rpm and temperatures as 25, 30, 35, and 40°C were experimented.

Determining the Dyes and Decolorization Environments with "Brine-shrimp Toxicity" Test

At the end of the decolorization studies conducted in optimum conditions, culture environments were centrifuged and the toxicity of culture supernatants were found out with *Artemia salina* toxicity test. Also, the toxicity of dye solutions, which were not processed, was determined. Rock salt was solved in water. Rock salt is the environment where *Artemia salina* larvae can grow. Each dye different concentrations ranging between 25-1000 ppm were prepared and this was poured into tubes where there is 5 ml solution. From the areas where there is intense larvae population provided to move to the light, with the help of micropipette set to 10 µl, the larva taken with water, dropped into the empty petri dishes under the stereomicroscope and 10 *Artemia salina* were counted. Then to the dye tubes, which have different concentrations with 5 ml salted water and control tube in which there are only salted water, 10 larvae were transferred. At the end of 24 hours, again by using

stereomicroscope dead larvae were counted and recorded. Data was evaluated with Probit Analysis Computer program and with LD50 values their 95% reliability limits were calculated. The studies were conducted double parallel.

Statistical Analysis

The effects of different environment conditions on decolorization rates were evaluated statistically. The differences created on the decolorization by the changed environment conditions (pH, dye concentration, shaking speed, and temperature) were statistically analyzed by the SPSS program.

RESULTS

In this study, the most appropriate conditions for the destruction of the reactive dyes frequently used in the textile industry was determined. Therefore, the effects of parameters like pH, dye concentration, shaking speed, and temperature were determined.

Determining Optimum Conditions for Decolorization pH

In the medium, where pH was set to 3.5-6.5 and dyestuff added, the rates of decolorization done by *P. sajor-caju* depending on time was checked. At the end of the studies conducted with *P. sajor-caju* fungi, decolorization percentage was higher in the environments where pH was 4.5. Againat 4.5 pH the speed of dye decolorizing was considerably higher if compared with the pH 3.5, 5.5, and 6.5.

In the condition when the medium of *P. sajor-caju* fungi was set to 4.5 pH, within the first 24 hours 50% decolorization happened, at pH 3.5, the rate of decolorizing dye was stable at the rate of 14-27%, at 5.5 pH Blue 49 51%, RBBR 58% decolorizing was determined. Similarly, at pH 6.5 it showed low decolorization rate staying between 18-29% (Fig. 1).

The study, which was carried out with *P. sajor-caju* at the 4.5 pH, a higher decolorization percentage ($p<0.05$) was obtained than the other pH applied.

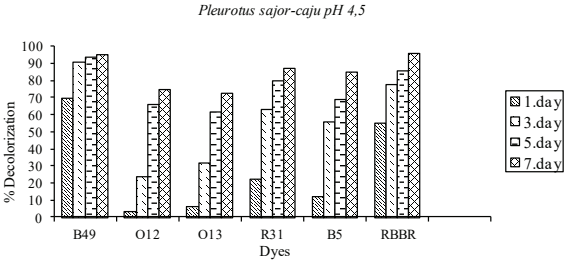

Figure 1. Time course of dye decolorization by *P. sajor-caju* at pH 4.5

Dye Concentration

Dyestuff added 25, 50, and 100 mg/l medium of *P. sajor-caju* fungi as a result of the decolorization the more the concentration of dyestuff in the textile dyes, the less decolorization.

Within 24 hours in the medium, which were added 25 mg/l Blue 49 and RBBR 78% - 72%, in 50 mg/l 73% - 65% and in 100 mg/l 59% rate of decolorization happened. The more dyestuff concentration added, the less decolorization rate observed. However, when the values were checked at the end of the incubation period, it was seen that the decolorization rates were considerably close to each other. Therefore, the change of dyestuff concentration did not affect the total decolorization rate substantially but it affected the decolorization speed. Statistically, between 25 and 50 mg/l concentrations a meaningful difference was not determined.

At the end of the incubation of 5 days, by *P. sajor-caju* fungi including 25 mg/l Orange 12 and Orange 13 71% decolorization rate was reached. As the dyestuff substance concentration increased, the speed of decolorization got low. When the total decolorization rates were checked the medium in which 100 mg/l dyestuff was added, the rate of being decolorized was low if compared to the other concentrations. The reason why decolorization speed and rate got low is the increase of dyestuff concentration. When Orange 12 and Orange 13 was raised from 25 mg/l to 100 mg/l *P. sajor-caju* fungi had a statistically meaningful difference.

In the medium where 25 mg/l of Red 31 added 97% decolorization, at the end of the 7 days of incubation 93% decolorization rate was reached in the medium, which was added 50 mg/l. Decolorization rates are almost similar to each other. However, in the medium containing 100 mg/l dyestuff concentration, at the end of the seven days period of incubation 49% decolorization was seen and this is lower than the other concentrations.

In Black 5 textile dye, as in the other dyes the more dyestufff, the slower decolorization. At the end of the 7 days in 25 mg/l 93% and 50 mg/l 90% rates were obtained and these are very close to each other. When it was raised to 100 mg/l, the rate decreased and 51% low decolorization was seen (Fig. 2).

Black 5 and Red 31 dyes in 25 mg/l and 50 mg/l concentrations, statistically any meaningful difference was not seen ($p<0.05$) but in 100 mg/l when compared with the rate of other concentrations, a meaningful difference was determined.

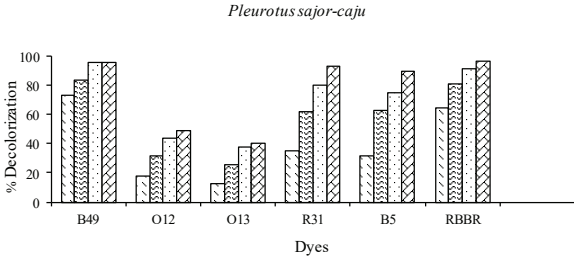

Figure 2. Time course of dye decolorization by *P. sajor-caju* at 50 mg/l dye concentration

Shaking Speed

For the decolorization by using different shaking speeds, it was researched if a higher decolorization could be reached by increasing the shaking speed or not. Three different shaking speeds were experimented.

As a result of the experiments carried out with *P. sajor-caju* fungi and Blue 49 and RBBR textile dyes, in the dye concentration of 50 mg/l at the speed of 200 rpm the decolorization was close to 150 rpm but faster. While at 200 rpm on the fifth day in Blue 49 96%, in RBBR 92%, at 150 rpm on the seventh day in Blue 49 95%, in RBBR 97% decolorization happened. At the speed of 100 rpm on the seventh day in Blue 49 63%, in RBBR 57% decolorization was observed.

In the concentration of 25 mg/l Orange 12 and Orange

13 textile dyes gave 59% and 61% on the fifth day at the speed of 150 but at the speed of 200 rpm on the fifth day both two dyes showed 71% decolorization.

In 50 mg/l of dye concentration Red 31 textile dye reached 62% on the third day at the speed of 200 rpm; on the seventh day 93% decolorization was reached. At the speed of 150 rpm on the third day 57%, seventh day 88% decolorization happened. At the speed of 100 rpm as a result of weekly incubation 53% decolorization happened.

In 50 mg/l dye concentration Black 5 textile dye gave 90% decolorization at the speed of 200 rpm; 85% decolorization at the speed of 150 rpm and these results, which are close to, each other were taken. At the speed of 100 rpm 50% decolorization, which is low compared with others, was seen (Fig. 3).

As a result of the experiments with different shaking speeds *P. sajor-caju* fungi showed a statistically meaningful difference compared to other shaking speeds; in the other shaking speeds a meaningful difference was not seen ($p<0.05$)

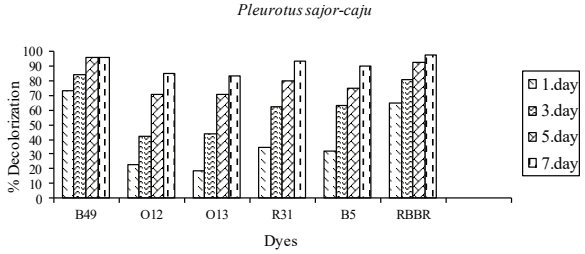

Figure 3. Time course of dye decolorization by *P. sajor-caju* at 200 rpm shaking speed

Temperature

For the decolorization, by using different temperature degrees (25, 30, 35, and 40), with the increasing temperature as a result of the studies of which more decolorization efficiency could be reached or not, *P. sajor-caju* fungi showed the best decolorization at 30°C.

With *P. sajor-caju* fungi experiments were carried out at different temperatures and the best result was taken at 30°C. Statistically a meaningful difference was taken compared to the other temperature degrees.

While Blue 49 dye showed 56% decolorization at 25°C in 24 hours, at 30°C in 24 hours 73%, at 35°C 44%, at 40°C 25% decolorization was reached. As it is seen in the results the best decolorization resut was taken at 30°C.

RBBR textile dye like Blue 49 gave its best result at 30°C. On the seventh day at 30°C 97% decolorization result was reached. Within 24 hours 65% dye was removed. As a result of one week of incubation at 25°C 69%, at 35 °C 54%, at 40°C 29% decolorization was seen.

At the end of the one week of incubation, Orange 12 and Orange 13 textile dyes at 25°C showed 35% and 39%, at 30°C 85% and 83% decolorization rates which are close to each other. However, at 35°C 33% and 29%, at 40°C 21% and 19% decolorization results which are low were reached.

62% decolorization was observed for Red 31 textile dye at 30°C on the third day of incubation. When the temperature was increased to 35°C, on the third day 32%, at 40°C it was 21%. As the temperature got higher than 30°C, decolorization dropped down. At 25°C at the end of three days of incubation 44% decolorization was reached (Fig. 4)

For Black 5 textile dye the most appropriate temperature is 30°C. At the end of 7 days of incubation 90% decolorization, at 25°C 58%, at 35°C 51% and 40°C 25% decolorization was observed.

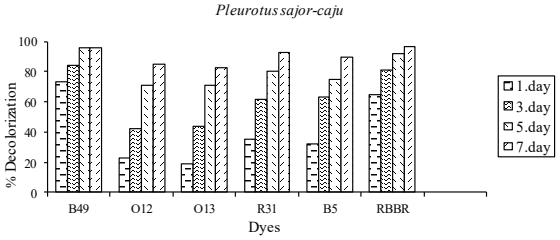

Figure 4. Time course of dye decolorization by *P. sajor-caju* at 30°C incubation temperature

The Toxicity of Decolorization Products

In order to determine the toxicity of the products, which are the results of the reactive dyes used in the study and their degradation, *Artemia salina* acute toxicity test was conducted. According to this all the dyes were determined as harmful. It was also determined if the products obtained from the decolorization studies which were carried out under the optimum circumstances were toxic or not. The data collected from the toxicity test was calculated by using a computer program called Probit Analysis and with their LD50 values their top and bottom 95% reliability limits were revealed. Toxicity test results were given in Tab. 2. The toxicity evaluations of the results were done according to the reference values in Tab. 1.

Table 1. The reference values used in the toxicity level evaluation [5].

Toxicity Level	LD50 Limits
Quite Toxic	<10 (µg/ml)
Toxic	>10 (µg/ml)
Harmful	>100 (µg/ml)
Non Toxic	>1000 (µg/ml)

Table 2. Brine-Shrimp Toxicity Assay Test Results of the dyes and LD50 values

Dyes	LD_{50} (µg/ml)	Upper %95 Reliable Lim.(µg/ml)	Under %95 Reliable Lim.(µg/ml)	Toxicity degree of decolorization environment
Blue 49	578.15µg/ml	472.88 µg/ml	774.08 µg/ml	Non Toxic
Orange 12	280.81µg/ml	214.33 µg/ml	387.86 µg/ml	Non Toxic
Orange 13	298.59µg/ml	243.12 µg/ml	376.04 µg/ml	Non Toxic
Red 31	425.67µg/ml	351.86 µg/ml	551.04 µg/ml	Non Toxic
Black 5	484.20µg/ml	390.61 µg/ml	660.54 µg/ml	Non Toxic
RBBR	527.26µg/ml	432.36 µg/ml	699.54 µg/ml	Non Toxic

DISCUSSION

Colored organic compounds have an important place among the factors causing environmental pollution. Contaminants containing dye are considerably disapproved aesthetically because of their colors. Textile and dye factories release contaminants to the receiving environment permanently and bound to this upon their potential polluting position the necessity of research increased more and more.

In this study, Reactive Blue 49, Reactive Orange 12, Reactive Orange 13, Reactive Red 31, Reactive Black 5, Reactive Blue 19, which are in the category of reactive dyestuff, with *P. sajor-caju* which is a white rot fungi, the effectiveness of pH, dye concentration, shaking speed and temperature on decolorization was researched.

In the studies related to pH maximum decolorization generally happened in the pH value where the microorganism showed an optimum improvement or the enzyme, which had a role in decolorization, showed an optimum activity. Also, in this study *P. sajor-caju* maximum decolorization pH was 4.5. In pH 5.5 a small amount of decolorization happened. However, in pH 3.5 and 6.5, decolorization was too low.

In a study conducted by Shahvali and friends (2000) and Shin and friends (1997) researching environmental factors, which are effective on the decolorization of textile, dyes with white rot fungi it was determined that maximum decolorization happened at pH 3. As it went up from pH 5, it was seen that decolorization rate went down. Shahyali and friends claimed that the reason could be osmotic changes and hydrolyzing effect.

At the end of the study we carried out at pH 4.5 the best decolorization competency by Swamy and friends (1999) among textile dyes Amarath, Remazol Black B, Remazol Orange, Remazol Brillant Blue, Reactive Blue and Tropaeolin O dyes and among decolorization experiments carried out with white rot fungi pH 4.5 giving the best result had a similarity.

In the dyestuff concentration amount each dye gave a different result. The amount of dye concentration was chosen as 25, 50, and 100 mg/l. As the dye concentration got higher, decolorization speed got low.

Murugesan and friends (2006) used biofilm technology to remove the colors of textile dyes. In these studies by using purified lactase enzyme from *P. sajor-caju*, decolorization of Reactive Black 5 was tested. At the end of the studies the effects of dye (25-100 mg/l) and incubation time (24-48 hours) on decolorization was researched. It was determined that in order to be removed at a maximum rate the amount of Black 5 had to be 62.5 mg/l and 36 hours.

Kapdan and friends (2000), in the decolorization study carried out with white rot fungi, it was stated that as the dye concentration amount got higher, it could show a toxic effect for the fungi. For all textile dyes we have been using, as the dye concentration amount went higher, the time necessary for the decolorization got longer.

With *P. sajor-caju* in the studies conducted in order to determine the percentages of decolorization in different shaking speeds at 200 rpm compared to others a higher decolorization percentage was determined.

Similarly, by Shahvali and friends (2000) and Assadi and friends (2001) and Yeşilada and friends (2003) at 200 rpm shaking speed decolorization giving a good result was determined.

Chagas and friends (2001) with *P. sajor-caju* and *P. chrysosporium* white rot fungi was found out that the decolorizaiton of Amaranth, new coccine and Orange G was at 200 rpm.

In these studies, as the shaking speed got more, between the cells and the medium because there were more mass and oxygen transfer, decolorization happens faster. Similar to this, the possibility of microorganisms touching the molecules was faster and more.

The experiment conducted with *P. sajor-caju* for the decolorization the optimum temperature was determined as 30°C and as the temperature got higher the percentage of decolorization dropped down.

In the studies related to the decolorization of textile dyes with *P. sajor-caju*, Toh and friends (2003) determined the temperature as 30°C. These results are also the same with ours.

In the study in which decolorization of textile effluents with *P. chrysosporium* at 25 – 40°C carried out by Assadi and friends (2001) it was reached that at 30°C and 35°C decolorization was better.

None of the supernatants obtained from decolorization environments were determined toxic. LD50 values between 100 – 1000 µl/ml, in respect to its top and bottom reliability limits, were within the limits but LD50 values which is over 1000 µl/ml was observed exceeding the limits [5].

As a result; textile sector is the second industrial branch after food industry in the scope of Turkish economy. Therefore, it has non-negligible contributions to the country's economy. It is a must to discharge the effluent coming out either from the dyeing or the other processes of textile sector and the pollution by purification.

In the studies carried out in recent years it is shown that especially with white rot fungi very high decolorization percentages could be obtained. White rot fungi destroys the dyestuff by using its enzyms during the decolorization; thus, they remove any problem that the dyestuff could cause.

For this reason, as a result of this branch of industry, because of their characteristic chemical structure it is very difficult for them to be destroyed biologically and in order to clean the color or remove their pollutant burden, the studies cleansing with white rot fungi for the effluents containing the azo dyestuff must be done intensely.

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