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Decolourisation of reactive textile dyes Drimarene Blue X3LR and Remazol Brilliant Blue R by *Funalia trogii* ATCC 200800

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Abstract

Decolourisation of reactive dyes Drimarene Blue X3LR and Remazol Brilliant Blue R by white rot fungi *Funalia trogii* was studied under static conditions. The effect of various conditions such as mycelial age, initial dye and glucose concentrations on decolourisation were also investigated. Decolourisation activity of *F. trogii* was compared with *Phanerochaete chrysosporium* known as test microorganism. It was found that 7-day-old cultures were more effective than 5-day-old cultures of *F. trogii* for decolourisation of these dyes. Decolourisations by *F. trogii* of both dyes were increased with glucose concentration decreasing. In contrast, decolourisations by *P. chrysosporium* were decreased. *F. trogii* decolourised 92–98% of both dyes within 4–10 h. However, *P. chrysosporium* partially decolourised (11–20%) these dyes during 10 days incubation period under the same conditions.

Introduction

Industrial dyes can be released into the environment from two major sources: as effluents from synthesis plants and from dye-using industries, such as textile factories (Rodrigues et al. 1999).

During textile processing inefficiencies in the dyeing process results in 10–15% of all dyes being lost directly to wastewater, which ultimately finds its way into the environment (Kirby et al. 2000).

It is reported that there are over 100,000 commercially available dyes with a production of over 7×10^5 metric tons per year. These dyes are classified as follows; direct, acid, reactive, basic, disperse, anthraquinone and metal complex dyes (Fu & Viraraghavan 2001).

Reactive dyes are very soluble by design and, as a result, not all are used up by textile fibers during the dyeing process and therefore end up with the discharge from dyehouses. They are hardly biodegraded under aerobic conditions (Mielgo et al. 2001; Panswad & Luangdilok 2000).

White rot fungi can degrade a wide variety of structurally diverse pollutants (Chagas & Durrant 2001; Swamy & Ramsay 1999; Yesilada et al. 2002). In recent years, many studies have demonstrated that the white rot fungi were able to decolourise dyes (Cripps et al. 1990; Kapdan & Kargi 2002; Kirby et al. 2000; Levin et al. 2003; Mazmanci et al. 2002; Podgornik et al. 1999; Selvam et al. 2003; Spadaro et al. 1992; Swamy & Ramsay 1999). Although most researches on dye decolourisation involve two fungi *Phanerochaete chrysosporium* and *Coriolus versicolor* which is also known as *Trametes versicolor* (Kapdan & Kargi 2002; Kapdan et al. 2000), *Funalia trogii* (*Trametes trogii*) degrades and decolourises recalcitrant effluents such as olive oil mill and alcohol factory wastewater and is therefore a good candidate for treatment of dyes and textile wastewater. There are a few studies on textile dye decolourisation ability of *F. trogii* (Yesilada et al. 2002, 2003; Mazmancı & Unyayar 2005).

Drimarene Blue X3LR (DMB, Reactive Blue 52) and Remazol Brilliant Blue R (RBBR, Reac-

tive Blue 19), which are widely used in textile industry in Turkey, have not been investigated for decolourisation by *F. trogii* before. In this study, decolourisation of these reactive dyes by *F. trogii* was investigated under static conditions and decolourisation activity of *F. trogii* was compared with *P. chrysosporium*, which has been examined for decolourisation of pulp mill wastewaters and various dyes by many researchers (Conneely et al. 1999; Couto et al. 2000; Fu & Viraraghavan 2001; Kapdan et al. 2000; Podgornig et al. 1999; Spadaro et al. 1992; Tatarko & Bumpus 1998).

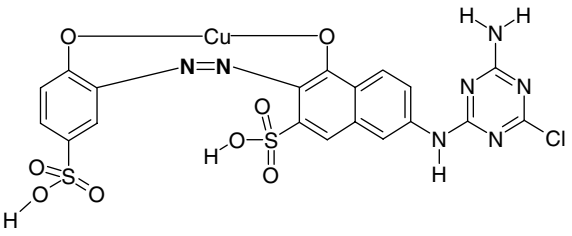
To determine the effects of initial dye concentrations on decolourisation of DMB and RBBR by *F. trogii* and *P. chrysosporium*, five different initial dye concentrations were studied and glucose concentration was fixed at 5 g/l.

Effects of glucose concentrations on decolourisation of DMB and RBBR decolourisation by *F. trogii* and *P. chrysosporium* in culture media, which contain 50 mg/l dye, were also investigated. Although some researchers reported that the presence of glucose increased decolourisation (Carliell et al. 1995; Kapdan et al. 2000), a few other studies did not observe this effect (Chen et al. 2003; Knapp et al. 1995; Özsoy et al. 2001). Swamy & Ramsay (1999) reported that approximately 0.1–0.3 g/l carbon concentrations were enough for decolourisation of Amaranth by *Trametes versicolor*. However, 5–40 g/l glucose concentrations were used in some of similar studies (Galhaup et al. 2002; Kapdan & Kargı 1999; Shin et al. 2002). If large amounts of glucose, e.g., 40 g/l, need to be added to textile effluents, which generally do not contain any glucose, then the cost of treatment will increase significantly. We wanted to see if less than 5 g/l glucose concentrations could provide similar decolourisation efficiency. For this aim glucose concentrations were chosen as 0.1–5.0 g/l.

Materials and methods

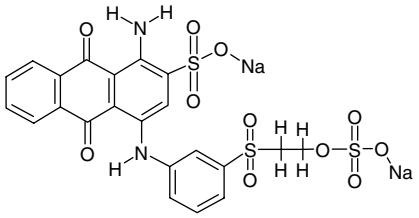
Dyes and chemicals

Commercial dyes Drimarene Blue X3LR (DMB, Figure 1A) and Remazol Brilliant Blue R (RBBR,



Molecular Weight = 631.49
Exact Mass = 631
Molecular Formula = C19H14ClCuN7O8S2
Molecular Composition = C 36.14% H 2.23% Cl 5.61% Cu 10.06% N 15.53% O 20.27% S 10.15%

(A)



Molecular Weight = 626.55
Exact Mass = 626
Molecular Formula = C22H16N2Na2O11S3
Molecular Composition = C 42.17% H 2.57% N 4.47% Na 7.34% O 28.09% S 15.35%

(B)

Figure 1. Chemical structures of dyes: (A) Drimarene Blue X3LR and (B) Remazol Brilliant Blue R [ISIS™/DRAW].

Figure 1B) were provided by Berdan Textile Co., Tarsus, Turkey. All other chemicals were obtained from Merck.

Microorganisms

Funalia trogii ATCC 200800 and *Phanerochaete chrysosporium* ME 446 were provided by Mersin University, Biotechnology Laboratory. Stock cultures were maintained on Potato Dextrose Agar plate transferred every 20 days and stored at +4 °C.

Cultivation conditions

Yesilada et al. (2002) reported that Astrazon type dyes were decolourised by *F. trogii* at various temperatures but maximum decolourisation efficiency was determined at 30 °C that is the optimum growth temperature of *F. trogii*. Similar decolourisation was observed at all tested pH values (6.0–11.0) (Yesilada et al. 2002). Based on these observations, we chose temperature of 30 °C, and pH of 6.0 for this study.

Cultures were inoculated slant agar and incubated at 30 °C for 5 or 7 days. After that, conidial and spore suspensions (10 ml, approximately 0.045 g dry weight) were used for inoculums. Ten millilitres of each suspension was transferred into 250 ml flask with 100 ml sterilised (1.2 atm, 120 °C, 15 min) stock basal medium containing 0.005 g/l $MgSO_4 \cdot 7H_2O$, 0.005 g/l $CaCl_2 \cdot 2H_2O$, 0.05 g/l $NH_4H_2PO_4$, 0.001 g/l $FeSO_4 \cdot 7H_2O$, 0.001 g/l $CuSO_4 \cdot 5H_2O$ and 0.1 g/l yeast extract. Medium pH was adjusted 6.0 with 0.1 M phosphate buffer.

The dye was diluted in sterile water and added to the flasks, right after they were inoculated with fungi. Culture medium was incubated at 30 °C.

Decolourisation determination

Samples were centrifuged at 5000 rpm, 20 min and supernatant was used for analysis. Experimental results are obtained by means of three replicates.

Absorbance measurements were done at maximum absorbance of dyes (609 nm for DMB; 614 nm for RBBR) by using a Shimadzu UV-160A Spectrometer. Decolourisation efficiency was determined as decolourised dye concentration/dried mycelium weight. Percentage decolourisation was calculated according to the following formulation:

$$\text{Percentage of decolourisation} = \frac{A_b - A_a}{A_b} \times 100$$

A_b is the absorbance at the maximum absorption wavelength of dye before decolourisation and A_a the absorbance at the maximum absorption wavelength of dye after decolourisation.

Two control groups were used in the assays. The first control contained only dyes at various concentrations. The second control contained fungus but no dye therefore provided a chance to compare toxic effects of dyes, if any, on test cultures.

In an attempt to solubilise any bound dye, the mycelia were homogenised in methanol and the homogenate was centrifuged and absorbance of the supernatant was then measured with spectrophotometer.

The dry weight of pellets was obtained by filtering cultures through filter paper and drying to a constant weight at 70 °C.

Anthrone reagent was used for determination of glucose concentration added in media. In this assay 1.0 ml of supernatant was mixed with 5.0 ml of a reagent containing 0.75 g anthrone in 1 l of 72% (v/v) H_2SO_4. The mixture was heated in a boiling water bath for 12 min, cooled to room temperature and measured the optical density at 620 nm. Glucose concentration was measured according to the method developed by Rosenberg (1980). Anthrone reagent was made fresh daily.

Enzyme assays

Laccase (E.C.1.10.32) enzyme activity in the media was assayed based on oxidation of guaiacol. Culture supernatant (1 ml) was added to 4 ml sodium phosphate buffer (pH 6.0), containing 10-μmol/l guaiacol and incubated at 30 °C. To assay the Peroxidase (E.C. 1.11.1.7) enzyme activity, 1.5 μl H_2O_2 was added to guaiacol solution and used same methods of laccase. Enzyme activities (1 U) were defined as the amount of enzyme that elicited an increase in A_{465} of 0,001 absorbance unit at hour (Fiskin et al. 1989; Hiroi & Eriksson 1976; Sık & Unyayar 1988). Data are means of three replicates.

Statistical analyses

For statistical analyses (ANOVA, REGRESSION, LSD and *T*-test) of experimental data, Statistica Software 6.0 was used.

Results and discussion

Effects of mycelial age

Five and seven day old cultures were used in determining the effects of mycelial age on decolourisation ability. Five-day-old cultures of *F. trogii* decolourised both dyes with approximately 90% efficiency within 120 h, however 7-day-old cultures reached this rate in 24 h. These results showed that the mycelial age of fungus played an important role on decolourisation process. Similar results were obtained by other researchers; the optimum age of mycelium was found six for decolourisation of dye Orange II (Knapp et al. 1997). Mazmancı & Unyayar (2005) were reported that different decolourisation activity of Reactive Black 5 was observed for different mycelia ages of *F. trogii*.

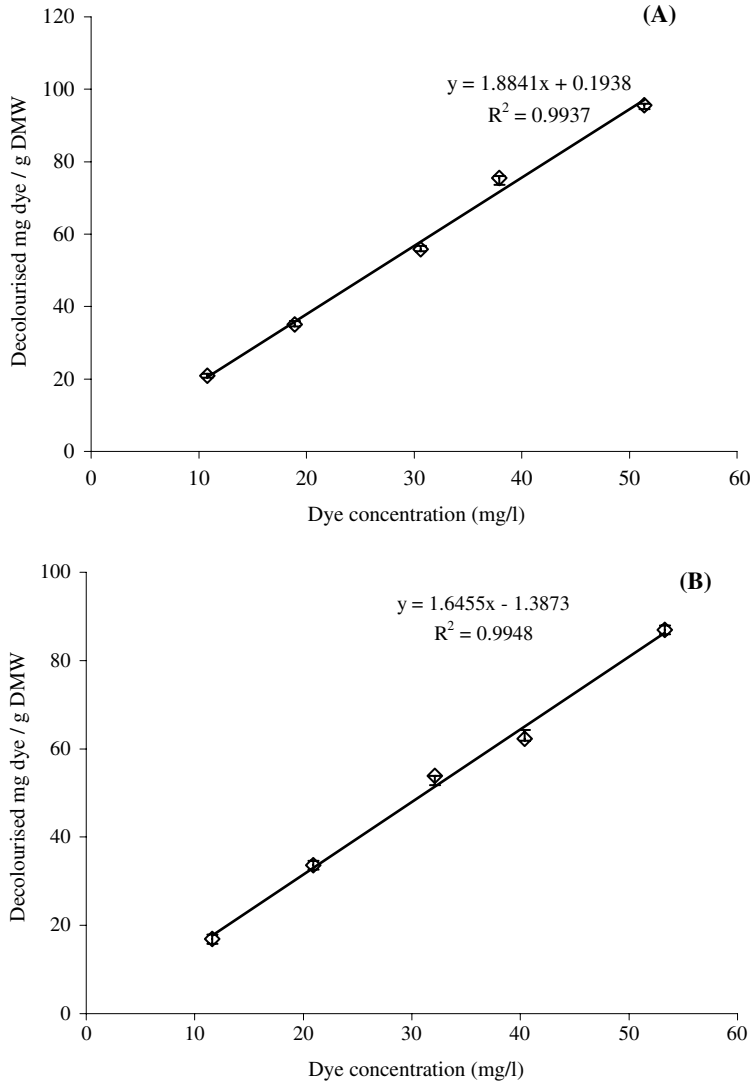


Figure 2. Dye decolourisation efficiency (decolourised amount of dye per unit dry weight of *F. trogii*) versus different dye concentrations. (A) Drimarene Blue X3LR (DMB), (B) Remazol Brilliant Blue R (RBBR). DMW: Dry mycelium weight.

Because 7-day-old cultures of *F. trogii* decolourised both dyes in 24 h, it was used for further experiments.

Effects of initial dye concentrations

The results showed that in all initial dye concentrations (10–50 mg/l), *F. trogii* could effectively decolourise DMB and RBBR dyes up to 90% within 24 h. However, *P. chrysosporium*, used as a test microorganism to compare with *F. trogii,* could decolourise only 11–20% of these dyes (10–50 mg/l) at the end of 10 days (240 h). These results indicated that decolourisation efficiency of *F. trogii* was higher than *P. chrysosporium* for these dyes. Figure 2 shows decolourisation ratio of dyes (decolourised mg dye/g dry mycelium weight) corresponding to initial dye concentrations. Plots of decolourisation ratio versus different initial dye concentrations are linear for both dyes within 10–50 mg/l dye concentrations (Figure 2). The slopes of these plots (1.8841 for DMB; 1.6455 for RBBR) indicate that, DMB decolourisation was higher than RBBR decolourisation. Also, Regression analyses results shows that p values of two plots are statistical significant ($p < 0.05$).

Spectrophotometric analysis of methanol extracts of *F. trogii* showed that the mycelium adsorbed less than 0.8% of DMB and 1.2% of RBBR after 24 h incubation. This shows that almost complete decolourisation of these dyes is mainly due to microbial metabolism and not to adsorption. Similar results were reported by Knapp et al. (1997) for Orange II decolourisation of fungus F29. However, several studies reported that the first step of microbial dye decolourisation was

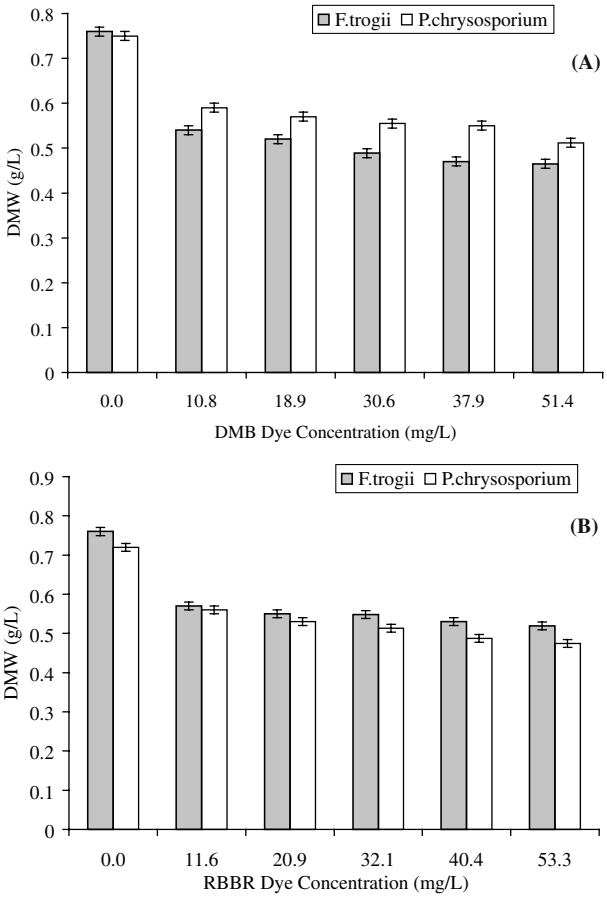


Figure 3. Dry mycelium weight (DMW) of *F. trogii* and *P. chrysosporium* after incubation for 24 h in different dye concentrations. (A) Drimarene Blue X3LR, (B) Remazol Brilliant Blue R.

adsorption (Jarosz-Wilkolazka et al. 2002; Knapp et al. 1995; Wang & Yu 1998). Yesilada et al. (2002) and Zheng et al. (1999) reported that the decolourisation process involved initial adsorption of Astrazon Red FBL and Poly R-478 on *F. trogii* and *Penicillium* mycelia. No decolourisation observed in control groups containing only dye.

Growth of *F. trogii* and *P. chrysosporium* in dye containing media were monitored and compared with control groups containing only fungi and no dye. ANOVA results indicate that differences between the dry weights obtained with different concentrations of both dyes are significant ($p < 0.05$). Figure 3 shows dried mycelium weight versus dye concentration, which clearly indicates that toxic effects increase along with increasing dye concentration in media. The data suggest that both DMB and RBBR showed toxic effects on growth of *F. trogii* and *P. chrysosporium*, although DMB was more toxic than RBBR for *F. trogii*. In parallel to our results, several past studies also reported toxicity and genotoxicity of textile dyes (Al-Sabti 2000; Chen 2002; Gottlieb et al. 2003; Marlasca et al. 1998; Rosa et al. 2001; Walthall & Stark 1999; Yesilada et al. 2003; Yun & Qi-xing 2002). These past studies collectively showed that cell concentration gradually decreased while dye concentration increased, a conclusion same as ours.

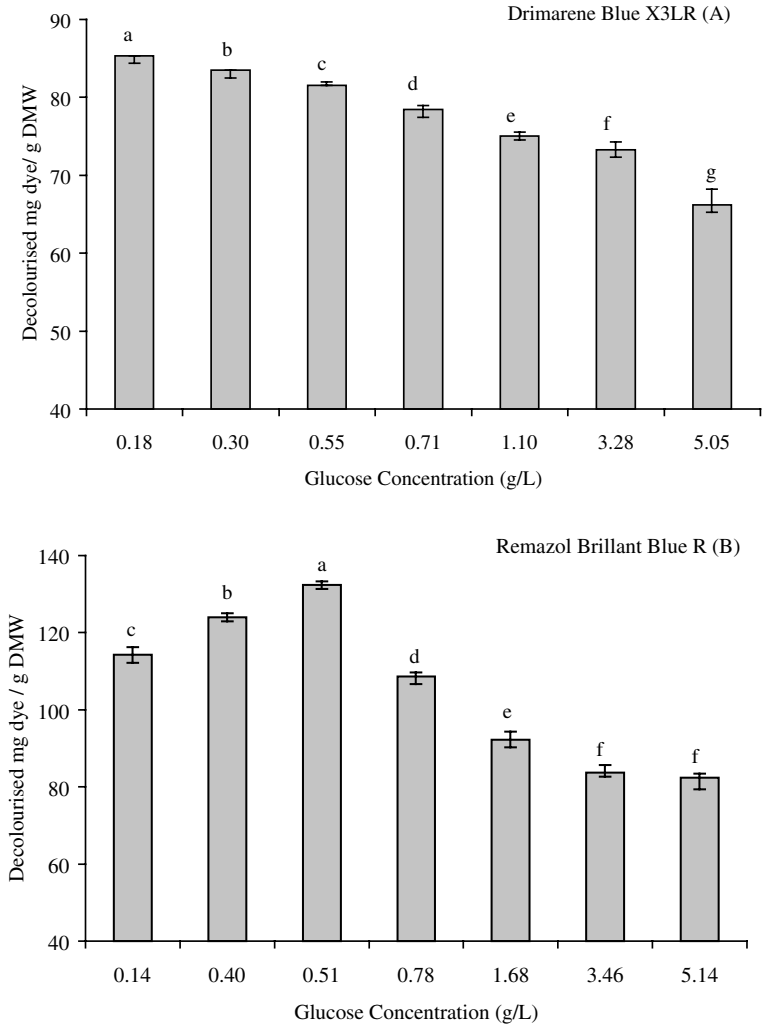


Figure 4. Effect of initial glucose concentration on decolourisation efficiency (decolourised amount of dye per unit dry weight of *F. trogii*) and LSD test results. (A) DMB – Drimarene Blue X3LR; (B) RBBR – Remazol Brilliant Blue R. DMW: Dry mycelium weight.

Effects of initial glucose concentrations

We found that decolourisation efficiency of both fungi was affected by glucose concentration in growth medium. When *F. trogii* was supplied with DMB, decolourisation efficiency decreased with increasing glucose concentrations, and the optimal glucose concentration was found to be 0.18 g/l. For RBBR, maximum decolourisation was achieved at 0.51 g/l glucose concentration (Figure 4). It was determined that there are significant differences in decolourisation efficiencies that result from varying the glucose concentration ($p < 0.05$). LSD test value of DMB and RBBR was determined as 0.1125 and 1.8340 respectively. These results indicated that optimum glucose concentration changed for *F. trogii* depending on the type of dye.

Decolourisation by *P. chrysosporium,* a well-studied model microorganism, increased with increasing glucose concentration reaching maximum decolourisation for both of dyes at glucose concentration of 5 g/l (Figure 5).

In accordance with previous studies, our experimental results show that decolourisation efficiency was affected by type of fungi, glucose concentrations of media, and the type of dye (Fu & Viraraghavan 2001; Kapdan et al. 2000a, b; Novotny et al. 2001).

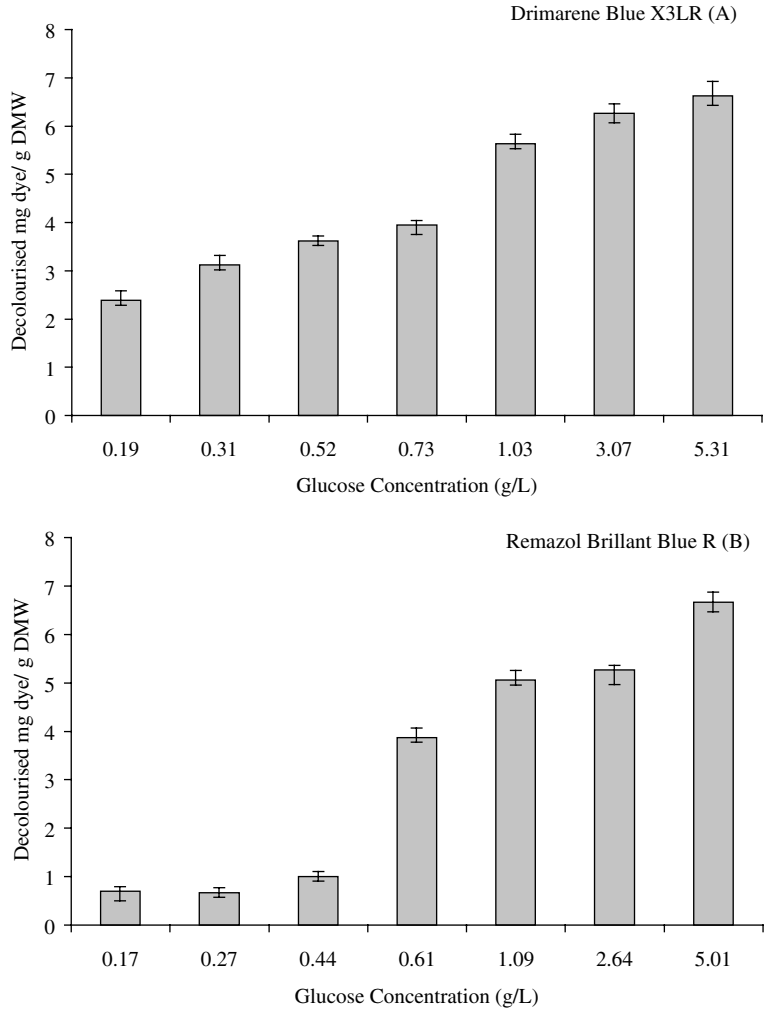


Figure 5. Effect of initial glucose concentration on decolourisation efficiency (decolourised amount of dye per unit dry weight of *P. chrysosporium)*. (A) DMB – Drimarene Blue X3LR; (B) RBBR – Remazol Brilliant Blue R. DMW: Dry mycelium weight.

Determination of optimum decolourisation time

In the previous sections, we determined the optimal mycelial age, and optimal glucose concentrations for decolourisation of DMB and RBBR by *F. trogii*. In addition, we also observed that 90% decolourisation occurred within 24 h, however, we wanted to find the optimal decolourisation time for both dyes with *F. trogii*. To achieve this goal, the experimental system was supplied with initial dye concentrations of 50, 100, 200 mg/l, and samples were taken every 2 h. For DMB, optimal decolourisation times by *F. trogii* were 4 h (50 mg/l, 92%), 8 h (100 mg/l, 92%) 10 h (200 mg/l, 98%). Similarly, RBBR was decolourised by this fungus within 8 h (50 mg/l dye, 87%), 10 h (100 mg/l dye, 90%), 10 h (200 mg/l dye, 94%) (Figure 6). *T*-test results indicate that decolourisation values for different concentrations of DMB are significantly different in 4 h. However, decolourisation of 100 and 200 mg/l in 8 h and 50–200 mg/l in 10 h are not showed statistical difference.

The enzymatic systems of white-rot fungi have not been fully characterised. It was reported that *F. trogii* excretes extracellular laccase enzyme different from *P. chrysosporium* (Garzillo et al. 1998). Our enzyme assays indicate that laccase and peroxidase enzyme activities in culture media of *F. trogii* were increased with increasing initial dye concentration. In culture media which contain 10 mg/l dye, laccase and peroxidase activities were determined as 0.020, 0.010 U for RBBR and 0.007, 0.007 U for DMB respectively. In the other media, which contain 50 mg/l dye, they were determined as 0.088, 0.050 U for RBBR and 0.068, 0.066 U for DMB.

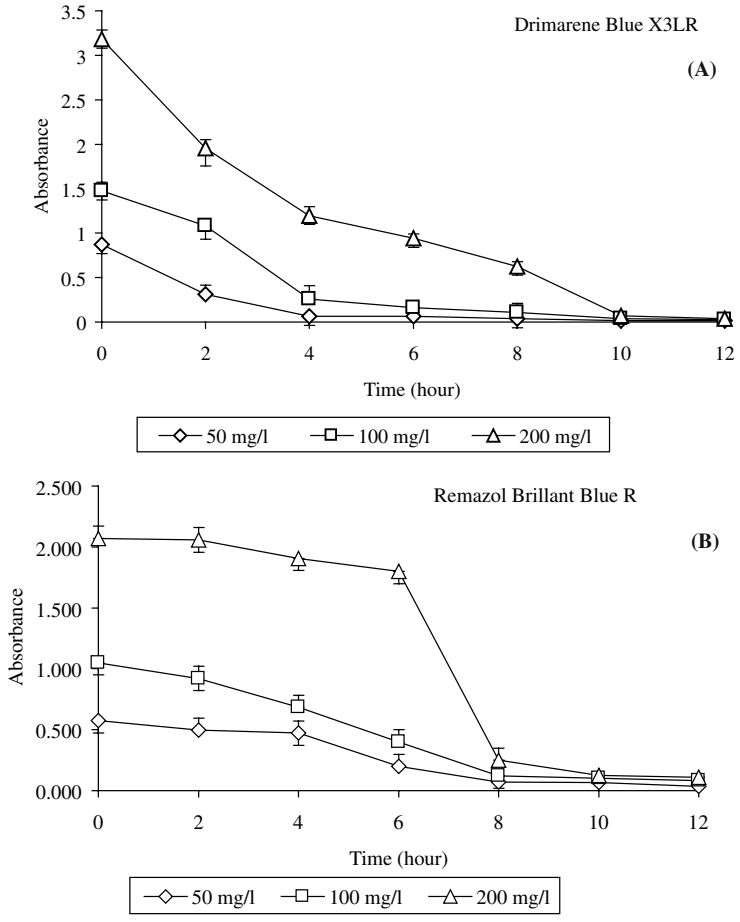


Figure 6. Decolourisation of Drimarene Blue X3LR (A) and Remazol Brilliant Blue R (B) by *F. trogii* at optimum glucose concentrations (0.1 g/l for DMB; 0.5 g/l for RBBR).

Conclusions

Effects of mycelial age, initial dye concentration and initial glucose concentration on decolourisation of reactive dyes Drimarene Blue X3LR and Remazol Brilliant Blue R by white rot fungi *Funalia trogii* was investigated and compared with *Phanerochaete chrysosporium*. Optimal mycelial age for decolourisation of these dyes by *F. trogii* was 7-day and initial glucose concentrations were 0.18 g/l for DMB and 0.51 g/l for RBBR.

P. chrysosporium partially decolourised (11–20%) Drimarene Blue X3LR and Remazol Brilliant Blue R during 10-day incubation period. On the other hand, *F. trogii* decolourised 92–98% of both dyes within 4–10 h.

Our results indicate that the white rot fungus *F. trogii* could effectively be used as an alternative to physical and chemical treatment processes that used for textile effluent treatment.

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