African Journal of Biotechnology Vol. 9(39), pp. 6539-6544, 27 September, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Decolorization efficiency of *Funalia trogii* under static condition: Effect of C: N ratios

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Accepted 18 August, 2010

Effects of physical conditions (pH and temperature), carbon and nitrogen source on decolorization of Reactive black 5 (RB5) by *Funalia trogii* were investigated under the static condition. Optimization of temperature and pH were also examined. Moreover, two different carbon sources (sucrose and starch), four different nitrogen sources and also four different C:N ratios (0.9, 3.0, 6.0, 18.0) were studied. Decolorization was expressed by mg dye / g dry mycelium weight. Optimal pH and temperature were found to be 4.78 and 30ºC, respectively. Decolorization efficiency increased with decreasing C:N ratio in starch-$NH_4H_2PO_4$, starch-urea, sucrose-$NH_4H_2PO_4$ and sucrose-urea containing media. Maximum decolorization was found as 9.61 and 7.77 mg dye/g dry mycelium weight in media containing no extra carbon and nitrogen sources. Kinetic studies were also carried out. The Lineweaver-Burk plot gave a Km of 406.66 mg/L and Vmax of 196.07 mg/L day for the diazo dye Reactive Black 5 decolorization by *F. trogii* under static condition.

Key words: *Funalia trogii*, decolorization, reactive black 5, static condition.

INTRODUCTION

At least 10% of total textile dye production or about 700 000 tones of dye per year is released into process waters (Wong and Yu, 1999; Vaidya and Datye 1982). Present physico-chemical treatments are expensive, and could generate a large volume of sludge. Dye effluents are poorly decolorized by conventional biological wastewater treatment (Shaul et al., 1991; Dubrow et al., 1996). As regulations become more stringent, the need for innovative, efficient and economic processes to treat these effluents increases. As a consequence, there has been a growing interest in biotechnological processes. White rot fungi produce non-specific, lignin-degrading enzymes which degrade a wide range of organic pollutants including textile dyes (Glenn and Gold, 1983; Heinfling et al., 1997; Ollika et al., 1993; Wesenberg et al., 2002; Deveci et al., 2004; Unyayar et al., 2005). Optimization of media plays an important role on decolorization processes whether fungal or not. Also, different decolorization rates of dyes result from different carbon and nitrogen sources used for fungal growth in dye containing media result. For this reasons, the best combination of carbon and nitrogen sources on fungal decolorization was still not known.

It was demonstrated in an earlier study that five different fungi (*Pleurotus chrysosporium*, *Pleurotus florida*, *Pleurotus eryngii*, *Pleurotus sapidus*, and *Funalia trogii* ATTC 200800) were tested, their decolorization abilities of reactive black 5 were also tested and the best decolorization activity was obtained from *F. trogii* cultures (Mazmanci and Ünyayar, 2005). In this study, we reported optimal pH and temperature and also ratio of carbon:nitrogen sources for decolorization process.

MATERIALS AND METHODS

Culture of *F. trogii* ATTC 200800 was obtained from Department of Mersin University, Biotechnology Laboratory. Stock cultures were maintained on Potato Dextrose Agar plate transferred every 20 days and stored at +4°C. Reactive Black 5 (Remazol Black B) were obtained from Turkey factories (Figure 1). The inoculums of fungus for liquid culture were prepared as follows: Five agar plugs of 1 cm^2 in diameter punched from the periphery of a seven-day agar plate

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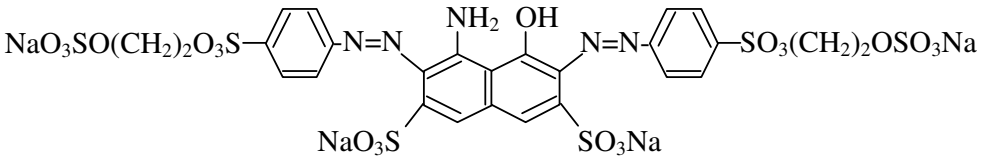

Figure 1. Structure of Reactive Black 5 (RB5).

Table 1. Preculture media.

Chemical	Amount (g/L)
$CaCl_2.2H_2O$	0.5
$MgSO_4.7H_2O$	0.5
$NH_4H_2PO_4/NH_4Cl$/Urea	0.05
Starch/sucrose	5.0
$MnSO_4$	0.025
$FeSO_4$	0.025
pH (phosphate buffer)	5.0

Table 2. Decolorization media.

Chemicals	Amount (g/L)
$CaCl_2.2H_2O$	0.5
$MgSO_4.7H_2O$	0.5
Dye (RB5)	0.0153*
pH (phosphate buffer)	5.0
C:N ratio	0.9, 3.0, 6.0 , 18.0

*Used for optimization studies.

were cultivated in a 100 ml bottle containing 30 ml preculture solution (Table 1).

Bottles were incubated in preculture media for seven days under static condition at 30°C and agitated for 5 min daily. The fungal mycelia were collected and homogenized with a politron homogenizerfor 30 s 9000 rpm under aseptic condition and 1 ml homogenized mycelia was inoculated into 30 ml decolorization media (Table 2). Optimization studies were studied at different temperature and pH. Different carbon (starch and sucrose), different nitrogen sources ($NH_4H_2PO_4$, NH_4Cl and urea) and four different C:N ratio (0.9, 3.0, 6.0, and 18.0) were tested in media after optimization studies (Table 3). Two control groups were used in the assays. The first control group contained dye but no inoculum. The second control group contained only 1 mL preculture medium that did not added extra carbon or nitrogen sources for determining preculture media effect.

Assays

Fungal hyphae were removed from a medium sample by centrifugation at 5000 x g for 10 min before assays. The concentration of Reactive black 5 (RB5) was determined by measuring its absorbance at 596.0 nm with a UV-Vis spectrophotometer (Shimadzu UV-Vis 160-A). Decolorization was determined spectrophotometrically by monitoring the decrease in absorbance at 596.0 nm, and calculated according to the following formulation:

$$\text{Decolorization efficiency (mg dye/g DMW)} = \frac{\text{Decolorized dye concentration}}{\text{Dry micelium weight}}$$

Table 3. Carbon and nitrogen sources used in decolorization experiment.

Carbon source	Nitrogen source	Symbol	Ratio
---	---	CNTRL	---
Starch/ Sucrose	$NH_4H_2PO_4$	A1	0.9
		A2	3.0
		A3	6.0
		A4	18.0
	NH_4Cl	B1	0.9
		B2	3.0
		B3	6.0
		B4	18.0
	Urea	C1	0.9
		C2	3.0
		C3	6.0
		C4	18.0

Fungal dry weight (DMW) was obtained by filtering the contents of each flask through pre-weighed Whatmann no. 1 filter paper and then drying to a constant weight at 103°C. Fungal dry weight was expressed as g of biomass per 1000 ml of culture. The concentration of glucose in the medium was estimated using the method of Rao and Pattabriman (Rao and Pattabiraman, 1989). Decolorization efficiency was expressed as decolorized mg dye per g dry mycelium weight. Results were the mean of the least three replicates.

RESULTS AND DISCUSSION

Four pH values (6.0, 5.5, 5.0, and 4.78) were tested to find the best initial pH for decolorization. Decolorization efficiency of *F. trogii* in media increased with decreasing initial pH value. Maximum decolorization was obtained in pH 4.78 media (11.20 mg decolorized dye/g dry weight) and the maximum fungal growth was determined in pH 5.0 medium (Figure 2). Kapdan et al. (2000) reported that pH 4.5 was the most suitable pH values for decolorization of dyes by *Trametes trogii*. However decolorization rate

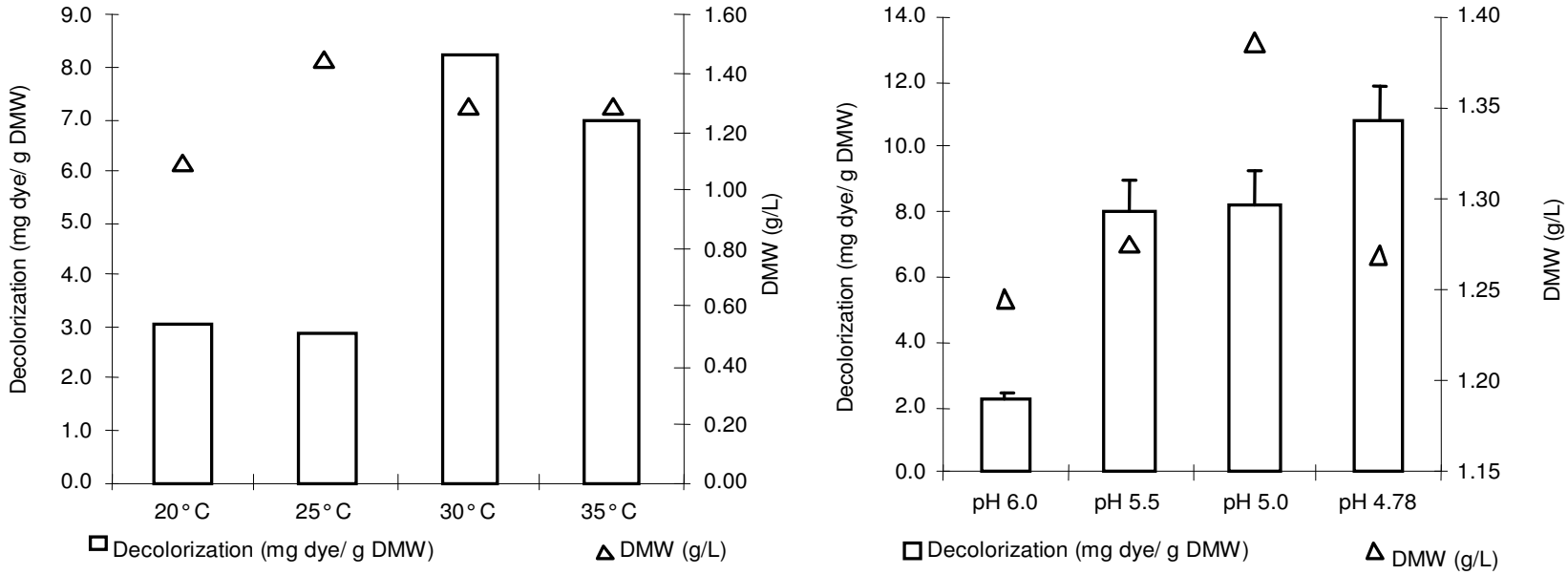

Figure 2. Effects of temperature and pH on decolorization and DMW.

of Astrazon Red FBL by *F. trogii* was found similar for tested pH values (pH 6 -11) (Yesilada et al., 2002). It was observed that pH was a little changed in all media except control at the end of six days (data not shown). This effect indicated that consumption of glucose concen- tration increased and the rate of organic acids in medium also increased (Chen et al., 2003).

Four different temperatures (20, 25, 30, and 35°C) were tested for decolorization. Decolori-zation efficiency was found higher on 30°C than other temperatures although maximum DMW was obtained from 25°C (Figure 2). Similar result was reported on decolorization of Astrazon red FBL by *F. trogii* (Yesilada et al., 2002). pH and temperature studies showed that optimal conditions for decolorization of Reactive Black 5 by *F. trogii* under static condition were 4.78 and 30°C, respectively. For this reason, further studies were performed in these conditions. In some decolorization experiments, fungi were grown in a media called preculture media to increase mycelial mass and later this mass were used as source of micro-organisms to decolorize dyes (Ozsoy et al., 2005; Mazmanci and Unyayar 2005). Researchers know that adaptation phase (lag phase) of microorganisms could be reduced when microorganisms are grown in preculture media and then inoculated in media containing same carbon sources. Authors, used same carbon sources in preculture and in decolorization media in order to reduce adaptation phase. Starch (textile wastewater may contain large quantities of starch resulting from the desizing process) and sucrose (find in molasses discharged from sugar industry) were used in decolorization experiments as carbon sources. Amounts of these carbon sources were fixed in decolorization experiments (1.8 g/L). It is known that the type and concentration of nitrogen source is an important parameter for degradation and/or decolorization process and also concentrations of sources affect the processes (Mazmanci et al., 2002; Nagarajan and Annadurai, 1999). In this study, three different nitrogen sources (ammoniumdihydrogenphosphate, ammonium chloride and urea) were used. C:N ratios were fixed at 0.9, 3.0, 6.0, 18.0.

Effects of starch containing media

Results of experiments for different nitrogen sources and concentrations in starch containing media are shown in Figure 3. Decolorization effi-

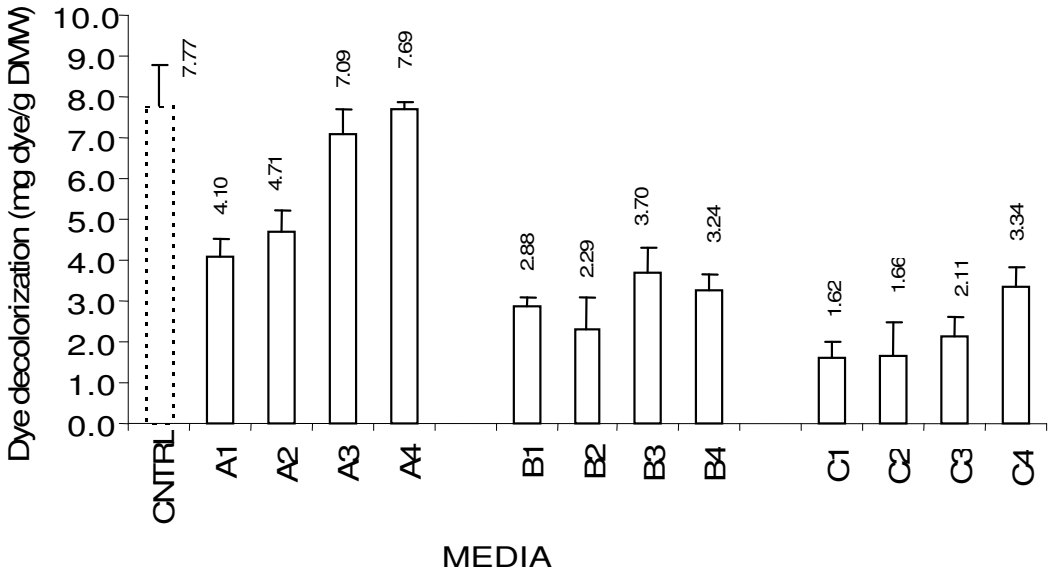

Figure 3. Decolorization efficiency of *F. trogii* in starch containing media.

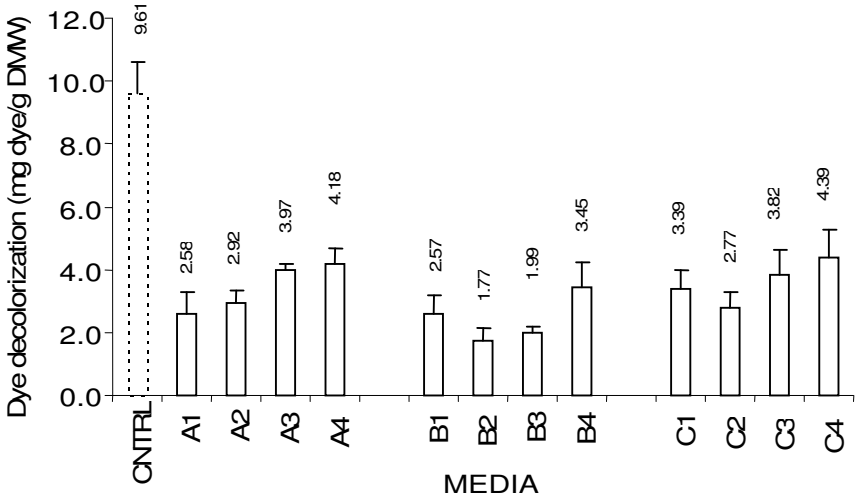

Figure 4. Decolorization efficiency of *F. trogii* in sucrose containing media.

ciency of fungus increased with increasing the ratio of C:N sources. Maximum decolorization was detected in control group (CNTRL) which was grown in starch containing media in preculture (Figure 2). Similar result was found in A4 media which is containing $NH_4H_2PO_4$ as a nitrogen source.

Effects of sucrose containing media

Decolorization of RB5 in media containing sucrose as carbon source was shown in Figure 4. Maximum decolorization was obtained in media CNTRL group which was grown in sucrose containing media in preculture (Figure 2) (9.61 mg dye/g DMW). Nitrogen source concentrations tested in sucrose containing media decreased the decolorization efficiency and these concentrations was not affected by the decolorization efficiency in sucrose containing media. Control groups show maximum decolorization. Sucrose and starch control groups reached 9.61 and 7.77 mg dye/g DMW, respectively. On the other hand, nitrogen sources were found to effect decolorization process in starch containing media. Ryu and Weon (1992) investigated various organic and inorganic nitrogen sources to determine the most suitable nitrogen source for decolorization by *Aspergillus sojae* B-10. Sodium nitrate was the optimal nitrogen source and the highest color removal occurred with NH_4NO_3 at 1.8 g/L.

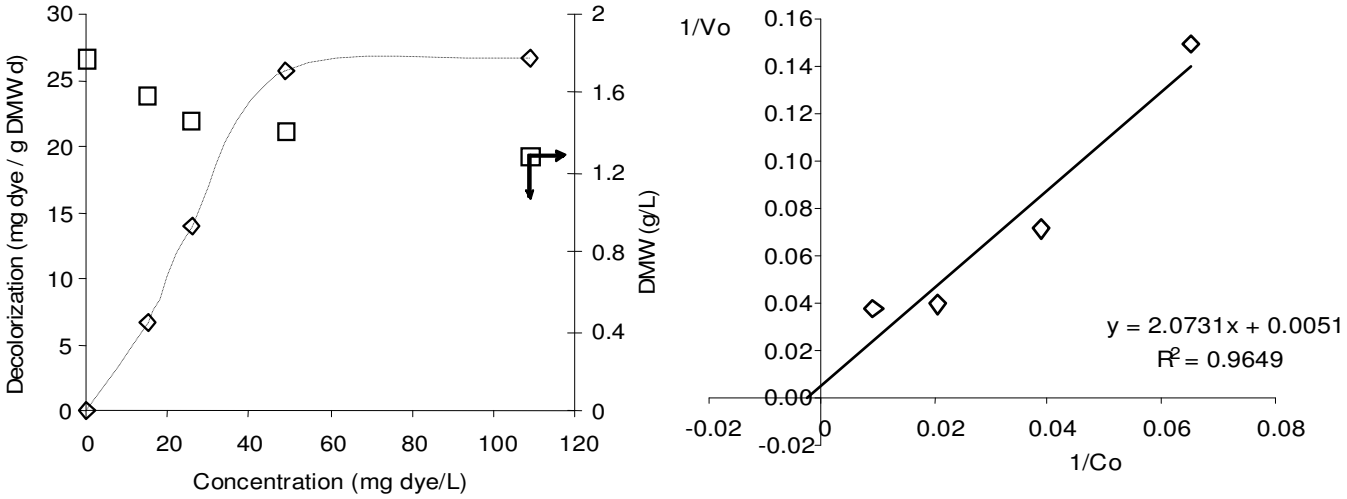

Figure 5. Experimental values of decolorization rate of RB5 using Michaelis-Menten kinetic model and Lineweaver-Burk plot of RB5 kinetic.

But Vasdev et al. (1995) reported that nitrogen had no effect on decolorization of dyes by *Cyathus bulleri*. The results showed that nitrogen source concentration affected the decolorization process and decolorization increased with decreasing nitrogen source concentrations in starch containing media. Tatarko and Bumpus (1998) also reported that addition of supplemental nitrogen only inhibited decolorization of Congo red in plates containing high amount of nitrogen source.

Mou et al. (1991) studied the effects of glucose concentration on decolorization of dyes by *Myrothecium verrucaria* and observed that the influence of glucose concentration was not significant to the biodegradation process. Belsare and Prasad (1988) observed that different decolorization efficiency of *Schizophyllum commune* with different carbon sources (sucrose, glucose, cellulose and pulp). On the other hand, Ozsoy et al. (2005) reported that optimum concentrations of glucose for decolorization of Drimaren Blue and Remazol Brillant Blue R by *F. trogii* were 0.18 and 0.51 g/L, respectively. Our results indicated that N-limited media containing starch played an important role on decolorization process. On the other hand, control groups which had no additional carbon and nitrogen sources were also found to be sufficient for decolorization.

Kinetic studies

In this study, we used low dye concentration to determine the optimal conditions such as pH, temperature, C-N sources and N source concentrations for dye decolorization. In order to determine the maximum decolorization rate of *F. trogii* in optimal conditions, different initial dye concentrations (15.3, 25.8, 49.0, 109.4 mg/L) were used. Conditions were fixed at 30°C, pH 4.78, C and N sources was not added in decolorization media and incubation time was determined six days. In this part of the study, fungus was grown in preculture media containing sucrose and $NH_4H_2PO_4$. DMW of *F. trogii* in dye containing media was monitored and compared with control group containing only fungi and no dye. Results showed that RB5 have toxic effects on fungal growth and this effect increased along with increasing dye concentration in media. Several studies also reported toxicity and geno-toxicity of textile dyes (Ozsoy et al., 2005; Al-Sabti, 2000; Rosa et al., 2001; Chen, 2002; Gottlieb et al., 2003). Ramsay and Nguyen (2002) also reported that RB5 was moderately toxic.

Results showed that time required for complete decolorization for 15.3, 25.8, 49.0, 109.4 mg/L initial dye concentration was determined as 6, 6, 9 and 12 days, respectively. This time was detected longer when compared to previous study (Swamy and Ramsay, 1999). Swamy and Ramsay (1999) reported that complete decolorization of 60 ppm reactive black by *Bjerkandera* sp. BOS55, *P. chrysosporium* and *T. versicolor* was determined 4, 2, and 3 days, respectively.

At the end of the incubation, decolorization was calculated as 1.11, 2.33, 4.27 and 4.46 mg dye/g DMW d. in 15.3, 25.8, 49.0, 109.4 mg/L dye containing media, respectively. The kinetic parameters of decolorization was calculated according to the Michaelis-Menten equation (Figure 5). The maximum decolorization rate concentration (Vmax) and half rate concentration (Km) were estimated from Lineweaver-Burk plots (Figure 5). The Lineweaver–Burk plot gave a Km of 406.66 mg/L and Vmax of 196.07 mg/L day for the diazo dye Reactive Black 5 decolourisation by *F. trogii*.

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