Ann Microbiol (2010) 60:269–278
DOI 10.1007/s13213-010-0037-0

ORIGINAL ARTICLE

Biodegradation of reactive dyes using soil fungal isolates and *Ganoderma resinaceum*

Eman H. F. Abd El-Zaher

Received: 22 October 2009 / Accepted: 11 February 2010 / Published online: 25 March 2010

Abstract The potential of *Fusarium oxysporum*, *Penicillium lanosum*, *Penicillium chrysogenum*, *Aspergillus fumigatus* and *Ganoderma resinaceum* to effect the biodegradation of five reactive textile dyes, namely Blue 21, Orange 16, Yellow 160, Blue 16 and Black 5, was evaluated. Preliminary experiments tested the decolorization ability of the tested fungi in three different media at a dye concentration of 0.02%. It was found that *F. oxysporum*, *P. lanosum* and *G. resinaceum* had high efficiency for decolorization of Blue 21. In the case of *P. lanosum* and *F. oxysporum*, decolorization was achieved in a basal Czapeks medium containing 0.01% reactive Blue 21 and supplemented with 3% glucose and 0.3% sodium nitrate after 5 days at pH 4.5 at 30°C. However, with *G. resinaceum*, optimum dye decolorization was achieved after 7 days incubation at 30°C and pH 5. Dye accumulation in the tested mycelia and changes in the outer surface of the cell wall of three studied fungi were observed. In addition, the structural integrity of Reactive Blue 21 dye untreated and treated with the tested fungi was studied by IR analysis.

Keywords Reactive dyestuff · *Fusarium oxysporum* · *Penicillium lanosum* · *Penicillium chrysogenum* · *Aspergillus fumigatus* · *Ganoderma resinaceum*

Introduction

Synthetic dyes have complex aromatic molecular structures, making them very stable and difficult to biodegrade. Such dyes are used extensively in textile dyeing, paper printing, color photography, and in the pharmaceutical, food, cosmetic and other industries (Rafii et al. 1990). Over 100,000 dyes are commercially available, with a total production of over 7×10^5 tons per year (Fu and Viraraghavan 2001).

It has been estimated that 10–15% of dyes are lost in effluent during dyeing processes (Zollinger 1987). Major classes of synthetic dyes, including azo, anthraquinone and triarylmethane dyes, are toxic or even carcinogenic compounds with long turnover times (Hartman et al. 1978). Azo dyes are the most important group of synthetic colorants. They represent the largest class of dyes, and more than half of the dyes produced annually (estimated for 1994 worldwide as 1 million tons) are azo dyes (Stolz 2001). Dye waste water from the textile or dye stuff industries is one of the most difficult types of wastewater to treat because dyes have various synthetic origins and contain complex aromatic molecular structures that make them very stable and hard to biodegrade (Kim et al. 2004; Abou-Okeil 2005). Many physical and chemical methods, including adsorption, coagulation, precipitation, filtration and oxidation, have been used for the treatment of dye-containing effluents. Adsorption is the method most widely used at present due to its convenience and efficiency (Churchley 1994; Nigam et al. 1996). More recent studies have demonstrated that many bacteria are able to degrade azo dyes aerobically and anaerobically (Govindaswami et al. 1993; Basibuyuk and Forster 1997). Some microorganisms, including bacteria, fungi and algae, can degrade or absorb a wide range of dyes (Robinson et al. 2001). Current interest is now focused on the microbial biodegradation of dyes as a better alternative (An et al. 2002). With the exception of some white rot fungi that can decolorize dyes, reports on decolorization by yeast or other filamentous fungi through enzymatic processes are very limited (Yang et al. 2003).

E. H. F. Abd El-Zaher (✉)
Botany Department, Faculty of Science, Tanta University,
El-Giesh St,
Tanta, Gharbia 31527, Egypt
e-mail: eimanabdelzaher@yahoo.com

The reactive dyes pose the greatest problem in terms of color because, in both ordinary and hydrolyzed form, they are not easily degradable, and thus, even after extensive treatment, color may still remain in the effluent (Santhy and Selvapathy 2006). However, reactive dyes can be removed easily and conventionally by aerobic microorganisms (Panswad and Luangdilok 2000).

The present study focused on the isolation of fungal species that can degrade Blue 21, Orange 16, Yellow 160, Blue 16 and Black 5 with high efficiency, and compared their decolorization ability with that of the higher fungus *Ganoderma resinaceum*. Furthermore, conditions accelerating Blue 21 dye biodegradation were optimized. The fungal morphology of the most efficient decolorizing organism was studied in order to understand the mechanism of dye degradation. The IR spectrum of reactive Blue 21 after biological treatment with the tested organisms was also studied.

Materials and methods

Dyes and chemicals

Five reactive dyes were used in this study: Yellow 4GL (basic chromophore is Yellow 160), Orange 3R (basic chromophore is Orange 16), Turquoise Blue G (basic chromophore is Blue 21) and Brilliant Blue R especial (basic chromophore is Blue 16). All these dye stuffs have only one reactive group in alkali medium that changes to vinyl sulfone to make a covalent bond between the dye and cellulose. Black B dye (basic chromophore is Black 5) has two reactive groups from vinyl sulfone (Fig. 1).

All dyes were kindly supplied by the Cairo Dyeing Center in industrial zone A2, 10th of Ramada City, Cairo, Egypt.

Fungal isolates

Soil samples as a source of fungi were obtained from the site around a dyeing factory in Tanta, Egypt. Fungi were isolated by using the dilution plate method (Van Walbeek et al. 1968). A sample of 1 g soil was added to a 100 ml flask containing 10 ml distilled sterilized water and shaken vigorously. Serial dilutions were then prepared and 0.5 ml of an appropriate dilution was cultured on Czapeks agar plates incubated at 28°C for 7 days.

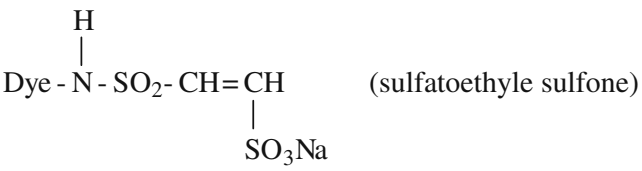

Fig. 1 Formula of reactive dye

Four fungal species were isolated (*Fusarium oxysporum*, *Penicillium lanosum*, *Penicillium chrysogenum*, and *Aspergillus fumigatus*) from the developed fungal colonies, identified according to Moubasher (1993) and tested for their ability to decolorize the five reactive dyes. Strains were maintained and subcultured on potato dextrose agar (PDA) slants or plates.

Isolation of *Ganoderma resinaceum*

Freshly collected *G. resinaceum* fruiting bodies were used to produce mycelia by cutting out the fresh fruiting body longitudinally using a sterilized knife (by flame and ethyl alcohol) then cutting transversely with sterilized forceps to pick up the fresh parts carrying the basidiospores of the hymenia . These were used to inoculate sterilized malt agar plates then incubated at 28°C for 9 days (Mustafa 2002). *G. resinaceum* was then cultured on three different media, as described below, to study its ability to decolorize the five tested reactive dyes.

Effect of medium and incubation time on decolorization efficiency of the tested fungi on the different reactive dyes

The effect of different liquid media and different incubation time (1–7 days) on the decolorization process was studied using the following media: potato dextrose broth (PDB), M1 medium [glucose 5 g, KH_2PO_4 1 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4{\cdot}7H_2O$ 500 mg and yeast extract 200 mg; Yang et al. 2003] at pH 5–6, and modified Czapeks broth (Ali and Muhammad 2008) containing per litre: sucrose 30 g, $NaNO_3$ 3 g, KCl 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $FeSO_4{\cdot}7H_2O$ 0.01 g and KH_2PO_4 1 g. Using a cork borer (0.5 cm), two discs for each tested fungus were inoculated in 250 ml conical flasks containing 100 ml sterilized liquid medium with 200 mg/l each tested reactive dye. The flasks were incubated at 25°C on a shaker at 140 rpm for different incubation periods (1–7 days). The experiment was repeated three times for each tested reactive dye on three media. Standard deviation was recorded for triplicate percentage decolorization readings (Yang et al. 2003).

Decolorization assays

Decolorization of dyes was detected using the culture supernatant after centrifugation at 3,000 rpm for 30 min. Control was performed using the same medium but without the tested microorganisms. Absorbance of the dyes revealed different λ max after scanning on a spectrophotometer (0–800; Table 1). All measurements were made on a

Table 1 Wave lengths (λ) of different tested dyes

Reactive dye	λ max (nm)
Blue 21	330, 658 and 619 nm
Orange 16	388 and 492 nm
Yellow 160	374 and 428 nm
Blue 16	285 and 592 nm
Black 5	313, 390 and 595 nm

Schimadzo UV visible recording Graphicord Spectrophotometer. Color removal was reported as:

$$\text{Decolorization } (\%) = (A_o - A_t)/A_o \times 100$$

were A_o is the absorbance of initial dye solution, which is constant for each dye and equals 0% decolorization, and A_t is the absorbance at cultivation time, t (Yang et al. 2003). Decolorization percentage reading refers to the percentage mean of decolorization percentage of three replicas.

Optimization of the decolorization process for reactive Blue 21 by the different fungi tested

Reactive Blue 21 (RB21) was selected for further investigation due to its potential for decolorization by *P. lanosum*, *F. oxysporum* and *G. resinaceum*. For optimization of the decolorization process, the fungi were grown on Czapeks liquid medium with added reactive Blue 21 for 5 days (*P. lanosum*, *F. oxysporum*) or 7 days (*G. resinaceum*). The effects of dye concentration (0.01, 0.05, 0.1, 0.15, 0.2% w/v), initial pH value (pH within the range 3–6), temperature (25, 30, 35, 40 and 45°C), different carbon sources (glucose, maltose fructose, starch and galactose were used instead of sucrose at 3% concentration), and different nitrogen sources (sodium nitrate, peptone, yeast extract and urea at 0.3% concentration) were studied.

Light microscopy examination

Morphological changes in the mycelia and spores of *P. lanosum*, *F. oxysporum* and *G. resinaceum* after treatment with reactive Blue 21 dye were assessed by light microscopy (magnification 40×).

IR analysis

After decolorization of Blue 21, the residual filtrate was dried. Dried reactive Blue dye was ground with KBr and analyzed (before and after treatment with the tested fungi) using Perkin-Elmer 377 at the Central Laboratory, Faculty of Science, Tanta University, Tanta, Egypt.

Results and discussion

Different physiological parameters were studied to optimize the process of dye biodegradation.

Effect of different media and incubation time periods

Three different media were used (PDB, M1 and Czapeks). The results in Tables 2, 3, 4, 5 and 6 show that *P. lanosum*, *P. chrysogenum*, *A. fumigatus* and *F. oxysporum* decolorized the five reactive dyes more efficiently on Czapeks medium than on PDB and M1 media.

Table 2 Decolorization percentage of five reactive dyes (0.02% w/v) in three different media by *Penicillium lanosum*. Each value is the mean ± SD of triplicate decolorization percentage. *CZ* Czapeks medium, *PDB* potato dextrose broth

Medium	Dye	Decolorization (percentage ± SD) at day:						
		1	2	3	4	5	6	7
CZ	Blue 21	65±0.1	67±2.08	72±2.5	77±2.5	82±1	70±2	68±2
	Orange 16	63±2.5	68±1	70±5	73±2	78±2	65±2	60±3
	Yellow 160	66±2	67±2.08	70±2.5	72±2.1	75±1	65±2	60±2
	Blue 16	63±2	65±1	71±2	75±1	78±1	70±3	69±2
	Black 5	50±1	56±2	60±5	62±3	64±2	57±2	50±2
M1	Blue 21	65±2	67±2	72±2.5	73±5	75±2	72±2	65±2
	Orange 16	63±5	65±3	67±2	68±2.5	72±3	65±2	60±2
	Yellow 160	55±1	60±1.5	65±2	67±3	70±1	65±2	60±2.1
	Blue 16	64±2	66±2.5	67±2	69±2	72±1	70±2	65±2
	Black 5	55±2.5	56±1	58±5	61±1	62±2.5	60±2	55±2
PDB	Blue 21	65±2	66±2	69±2.5	70±3	73±2	70±3	65±2
	Orange 16	56±2	60±2.5	62±2.1	65±1	69±2	65±2	60±2
	Yellow 160	56±2	64±3.2	65±2	66±5	67±2	60±2	55±3
	Blue 16	59±2.5	62±2	65±2	66±2.5	71±2	65±2	60±2
	Black 5	50±2.5	52±2	54±2.1	55±2.5	56±2	50±2	45±2

Table 3 Decolorization percentage of five reactive dyes (0.02% w/v) in three different media by *Fusarium oxysporum*. Each value is the mean ± SD of triplicate decolorization percentage

Medium	Dye	Decolorization (percentage ± SD) at day:						
		1	2	3	4	5	6	7
CZ	Blue 21	65±2	66±2	75±2.5	77±3	80±2	70±2	60±2
	Orange 16	45±2	60±1	65±2	70±3.2	75±2	70±3	56±2
	Yellow 160	55±2	65±2	70±2.5	72±1	73±2	66±2.1	60±2
	Blue 16	60±3	63±3	71±2	73±1	77±2.5	67±2	62±2
	Black 5	20±2	45±2	55±2.5	58±1	60±2	55±3	50±2
M1	Blue 21	66±2.5	67±2.1	69±3	70±3	73±2	70±2	62±2.1
	Orange 16	62±2	65±2	67±3	68±2.5	70±3	65±2	60±2
	Yellow 160	57±2	64±2.5	65±2.1	66±2	67±2	60±2	55±2
	Blue 16	60±2	61±2.5	63±2	65±3	70±2.5	65±2	60±2
	Black 5	45±2	49±2	50±3	55±2.1	59±2	50±2	45±2
PDB	Blue 21	60±2	65±2	66±3	67±2.5	70±2	60±2	56±2
	Orange 16	56±1	62±2	64±3	65±2	66±2.5	65±2	60±2
	Yellow 160	55±2	57±2	60±2	61±2	62±2	55±2	50±2
	Blue 16	56±2.5	58±3	59±2	62±2	65±2	60±2	55±2
	Black 5	47±2	49±2	50±3	52±2	56±2.5	50±2	45±2

The dye Blue 21 was decolorized more than the other four reactive dyes. The decolorization of Blue 21 dye increased with increasing incubation periods up to 5 days in case of *P. lanosum* (82%), *F. oxysporum* (80%), *A. fumigatus* (86%) and *P. chrysogenum* (70%) and declined thereafter, while in the case of *G. resinaceum*, the maximum decolorization was reached after 7 days (79%). It may be concluded generally that the composition of the medium affected growth and dye decolorization. Czapeks medium was chosen for subsequent experiments.

After decolorization, the peak wavelengths of reactive Blue 21 at 330 and 658 nm disappeared, and peaks for Orange 16, Yellow 160, Blue 16 and Black 5 at 388, 274, 285 and 595 nm, respectively, disappeared. These results indicated that decolorization percentages were high.

It was clear that decolorization of reactive Blue 21 dye by *P. lanosum* was more efficient than decolorization by *F. oxysporum* and *G. resinaceum*. Similar results were reported by Zheng et al. (1999) for *Penicillium* ATCC 74414 and *A. ochraceus*, which had the ability to remove color from poly

Table 4 Decolorization percentage of five reactive dyes (0.02% w/v) in three different media by *Ganoderma resinaceum*. Each value is the mean ± SD of triplicate decolorization percentage

Medium	Dye	Decolorization (percentage ± SD) at day:						
		1	2	3	4	5	6	7
CZ	Blue 21	67±2	72±1	75±2.1	76±2.5	77±2	78±2	79±2
	Orange 16	62±2	65±2	70±2	72±2	74±2	76±2	76±2.5
	Yellow 160	67±3	66±2	70±2	71±2	72±2	73±2	75±2.5
	Blue 16	65±2	67±2	72±1	75±2	76±3	77±3	76±2.5
	Black 5	25±2	45±2	55±2	56±1	57±2	59±2	60±2
M1	Blue 21	60±1	62±1	65±3	65±2.5	67±2	68±2	70±2
	Orange 16	57±2	60±2	63±2	64±2	64±2	65±2	66±2.5
	Yellow 160	56±3	58±4	59±2	62±2	63±2	64±2	65±2.5
	Blue 16	35±2	40±2	56±3	57±2	59±2	62±2	65±2.1
	Black 5	46±2	50±3	55±2.5	57±2.1	58±2	59±2	60±2
PDB	Blue 21	60±2	65±2.5	67±2.1	68±2.5	69±2	71±2	72±3
	Orange 16	55±2	62±1	54±0.1	65±5	65±2	66±2	67±2.5
	Yellow 160	55±1	58±3.2	60±3.2	60±2.5	60±1	61±3	62±3
	Blue 16	57±1	58±2	59±2	60±2	61±2	64±2	65±2.5
	Black 5	40±2	45±2	52±4	53±2	54±3	55±2	56±2.5

Table 5 Decolorization percentage of five reactive dyes (0.02% w/v) in three different media by *Aspergillus fumigatus*. Each value is the mean ± SD of triplicate decolorization percentage

Medium	Dye	Decolorization (percentage ± SD) at day:						
		1	2	3	4	5	6	7
CZ	Blue 21	66±2	65±4	69±2.1	75±2	76±2.5	70±1	65±2
	Orange 16	43±2.1	59±2	65±2.5	70±3	75±2	70±2	60±2
	Yellow 160	55±2	66±2.5	72±4	72±2	74±5	65±2	60±2
	Blue 16	45±2	55±2	60±2	67±2	75±2.5	70±2	60±3
	Black 5	45±3	50±2	54±4	56±3.2	62±4	60±2	50±2
M1	Blue 21	55±1	65±2	67±2	70±5	75±6	65±2	55±2
	Orange 16	57±2	59±2	65±2.5	68±2	70±4	57±2	55±2
	Yellow 160	52±1	55±5	57±5	64±2.5	65±2	60±2	55±2
	Blue 16	59±2	60±2.1	62±3	65±3	69±3	55±2	50±2
	Black 5	45±2	49±2	51±2	53±1	55±5	50±2	45±2
PDB	Blue 21	60±2	63±2	65±2.1	67±2.5	75±6	70±2	65±2
	Orange 16	56±2	62±1	65±2	66±2	70±2	69±2	66±2
	Yellow 160	62±2	64±2	65±2	68±2.5	72±2.5	70±2	61±3
	Blue 16	56±2	59±2	60±2.5	63±2.5	65±4	62±2	60±2
	Black 5	40±4	42±4	45±2	47±3	50±2	42±2	40±2

R-478 and reactive Blue 25, respectively. The possible mechanism of decolorization involved initial mycelial biosorption and subsequent degradation.

Husseiny (2008) found that the color reduction percentages of reactive and direct dye in the case of *Aspergillus niger* and *Penicillium* spp. were 86 and 82.66%, respectively, after 2 days, and reached a maximum reduction percentage after a 4-day incubation period.

Ali et al. (2009) also reported that decolorization of 50 μM malachite green by *A. flavus* and *Alternaria solani* increased to 96% by the 6th day in Czapeks medium. In the case of *Debaryomyces polymorphus* and *Candida tropicalis*, the decolorization percentage reached 94% and 97% of 100 mg reactive Black dye after 6 h (Yang et al. 2003). Ali and Muhammad (2008) reported that the decolorization percentage of acid violet 19 was 88.6% in the case of *Alternaria solani* after 4 days in modified Czapeks medium. Accordingly, Czapek's medium was chosen for subsequent experiments.

Effect of dye concentration on decolorization percentage of reactive Blue 21 with different test fungi

Figure 2a shows the effect of different dye concentrations on the decolorization percentage of reactive Blue 21 using

Table 6 Decolorization percentage of five reactive dyes (0.02% w/v) in three different media by *Penicillium chrysogenum*. Each value is the mean ± SD of triplicate decolorization percentage

Medium	Dye	Decolorization (percentage ± SD) at day:						
		1	2	3	4	5	6	7
CZ	Blue 21	35±2.5	45±2	55±2	66±5	70±4	65±2	59±2
	Orange 16	42±2	45±2	52±3	59±2	63±6	60±2	56±2
	Yellow 160	32±2.5	40±2.5	45±2.1	49±2	55±2	45±2	40±2.5
	Blue 16	45±2	49±2	52±2.5	56±2	62±2.5	55±2	50±2
	Black 5	30±2	32±2	35±2.5	39±3	40±4	34±1	30±2
M1	Blue 21	55±3	59±2	62±1	65±2	67±2.5	65±2	50±2
	Orange 16	45±5	47±5	52±4	62±2	66±2.5	60±2	55±2
	Yellow 160	35±3	40±2.5	47±2.1	49±2	55±2.5	50±2	47±2.5
	Blue 16	54±1	59±2	62±2.5	64±2.1	65±4	60±2	55±2
	Black 5	30±1	35±2	37±2	41±1	45±3	40±2	37±2
PDB	Blue 21	45±2	49±2	53±2.5	55±2.5	60±2	55±2	50±2.5
	Orange 16	35±2.5	42±2.1	45±4	49±2	56±3	50±2	45±2
	Yellow 160	37±1	40±2.5	42±5	45±4	50±2	45±2.1	42±2
	Blue 16	45±2.1	47±2	50±4	52±5	55±4	50±2	47±2
	Black 5	32±2.5	35±2	37±3	39±2	40±2	35±2	30±2

different test fungi. The maximum percentages of decolorization using *P. lanosum*, *F. oxysporum* and *G. resinaceum* were 88%, 87% and 85%, respectively, at a concentration of 0.01%. It is clear that 0.01% of reactive Blue 21 is the best concentration for high levels of decolorization. Our results are similar to results obtained by Bhatti et al. (2008) for *Shizophyllum commune* IBL-6, and by Zheng et al. (1999) for *Penicillium* strain ATTCC. Both fungi decolorized cibarcon red 2BL and poly R-478 at a concentration of 0.01%. Kapdan et al. (2000) concluded that dyestuff concentration should be lower than 0.05% for complete decolorization of Everzol turquoise Blue by *Coriolus versicolor*. Also, Yang et al. (2003) found that *Debaromyces polymorphus*, *Candida tropicals* and two filamentous fungi, *Umbelopsis isabellina* and *Penicillium geastrivorus* could decolorize 100 mg/L reactive Black completely within 16–48 h (Yang et al. 2003).

Effect of different pH

The results in Fig. 2b clearly show a gradual increase in dye decolorization by *P. lanosum*, *F. oxysporum* and *G. resinaceum* upon increasing the pH to 4.5 in case of *G. resinaceum* and pH 5 in the case of *F. oxysporum* and *P. lanosum*. However, a further increase in pH caused a slowly decolorization decreasing effect.

These results clearly support the concept that best fungal growth usually occurs at low pH values (Assadi and Jahangiri 2001). At higher pH values, reactive dye solutions are more negatively charged, and dye removal efficiency of white-rot fungi is decreased (Tak et al. 2004). It was clear from data reported by Husseiny (2008) that 93.73 and 96.69% decolorization of reactive and direct dye at pH 4.5 and 7, respectively, in the case of *A. niger* but 89.6% and 88.4 at pH 4 and 4.5, respectively, in the case of *Penicillium* spp.

Effect of temperature

We next studied the effect of different incubation temperatures on decolorization percentage of reactive Blue 21 dye. The results (Fig. 2c) showed that the tested fungi reached highest percentages of dye decolorization—89, 87 and 88% for *P. lanosum*, *F. oxysporum* and *G. resinaceum*, respectively—in shake flasks incubated at 30°C for 5 days, but *G. resinaceum* required 7 days. Indeed temperature has a considerable effect on many enzymes which control the biological reactions involved in growth, biosynthesis and decolorization of dye.

A decreasing trend of dye decolorization was observed at higher temperatures (40–45°C). White rot fungi show better growth under medium temperature conditions as compared to higher temperatures (Toh et al. 2003).

Husseiny (2008) reported decolorization of a direct dye bath by *A. niger* of 86 and 82.66% at 28 and 35°C, respectively, with the maximum decolorization percentage recorded after 4 days at temperatures of 28 and 35°C for reactive and direct bath effluent, respectively.

Effect of different carbon sources

As shown in Fig. 2d, glucose stimulated fungal growth and enhanced decolorization percentage to 92, 88 and 99% with *P. lanosum*, *F. oxysporum* and *G. resinaceum*, respectively,

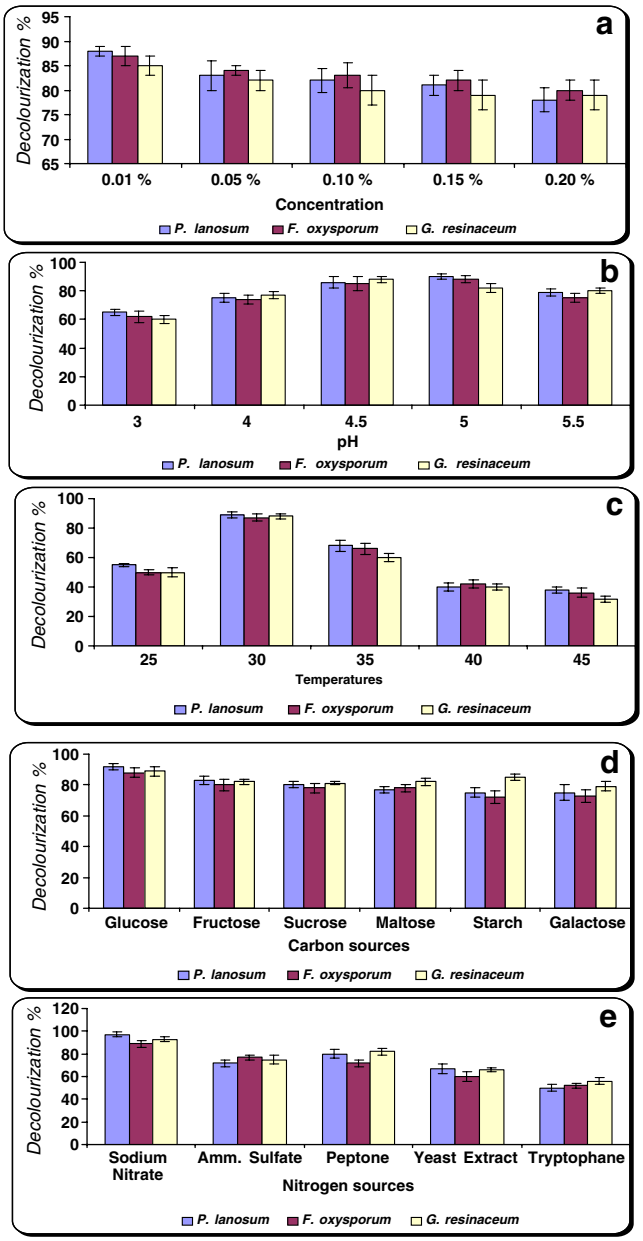

Fig. 2a–e Physiological factors affecting decolorization percentage with the test fungi. **a** Concentration of reactive Blue 21 dye, **b** pH value, **c** temperature, **d** carbon source, **e** nitrogen source. Values are mean ± SD of triplicate decolorization percentage

while fructose, sucrose, maltose and starch decreased decolorization compared with that of the control.

Glucose was used by the fungus as a readily consumable carbon source and it caused a significant shortening of lag phase. Glucose provides an easily metabolizable energy source to the fungus and creates the best environment to enhance the decolorization rate of dyes. Utilization of carbon sources and decolorization of dye are dependent on the enzymatic activity of the fungal species under study.

Decolorization percentage (94%) of cibacron red 2BL increased in medium supplemented with 1% glucose in the case of *Shizophyllum commune* IBL-6 (Bhatti et al. 2008). Shoda et al. (2000) found that using 10 g/L molasses increased reactive Blue 5 decolorization by *Geotrichum candidum* Dec1, but decolorization decreased at 20 g/L.

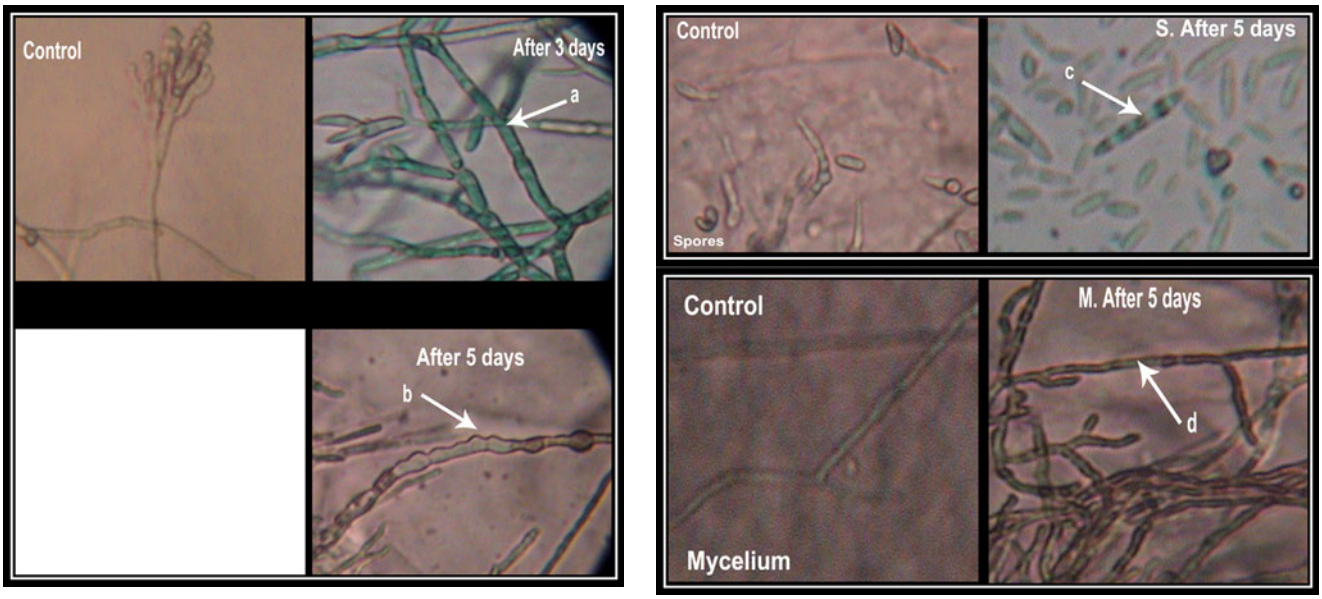

Fig. 3 Morphological characteristics of mycelia and spores of *Penicillium lanosum*, *Fusarium oxysporum* and *Ganoderma resinaceum* after treatment with Reactive Blue 21 dye. **a** Mycelium with Reactive Blue 21 color, **b** irregularly shaped mycelium, **c** macrospore septa with absorbed dye, **d** irregularly shaped mycelium, **e** spore with absorbed dye, **f** irregularly shaped mycelium

Similar results were found by Kapdan et al. (2000) with white rot fungi and Kapdan and Kargi (2002) with *Coriolus versicolor*—this fungus had ability to decolorize Everzol turquoise Blue G in the presence of glucose.

Effect of different nitrogen sources

Figure 2e clearly shows that the highest decolorization percentage (97%) was observed with 0.3% sodium nitrate in the case of *P. lanosum* and 93 and 89% with *G. resinaceum* and *F. oxysporum*, respectively.

Similar results were obtained by Ali et al. (2009), who found that *Aspergillus flavus* and *Alternaria solani* were able to decolorize (96%) of malachite green (10–50 μM) in Czapeks medium supplemented with 0.3% sodium nitrate. Lele et al. (2007) also reported that maximum decolorization percentage (96%) was recorded with 100 ppm amaranth dyes using *Ganoderma* sp.

Morphological changes in mycelia and spores after treatment with reactive Blue 21 dye

Penicillium lanosum absorbed the color of reactive Blue 21 after 3 days and showed irregularly shaped mycelium after 5 days (Fig. 3a). Macrospores of *F. oxysporum* accumulated the absorbed color of dye on septa, and the mycelium exhibited an irregular shape and wrinkled outer cell wall surface (Fig. 3b). Spores of *G. resinaceum* also absorbed the color of the dye and the mycelium showed an irregular shape (Fig. 3c).

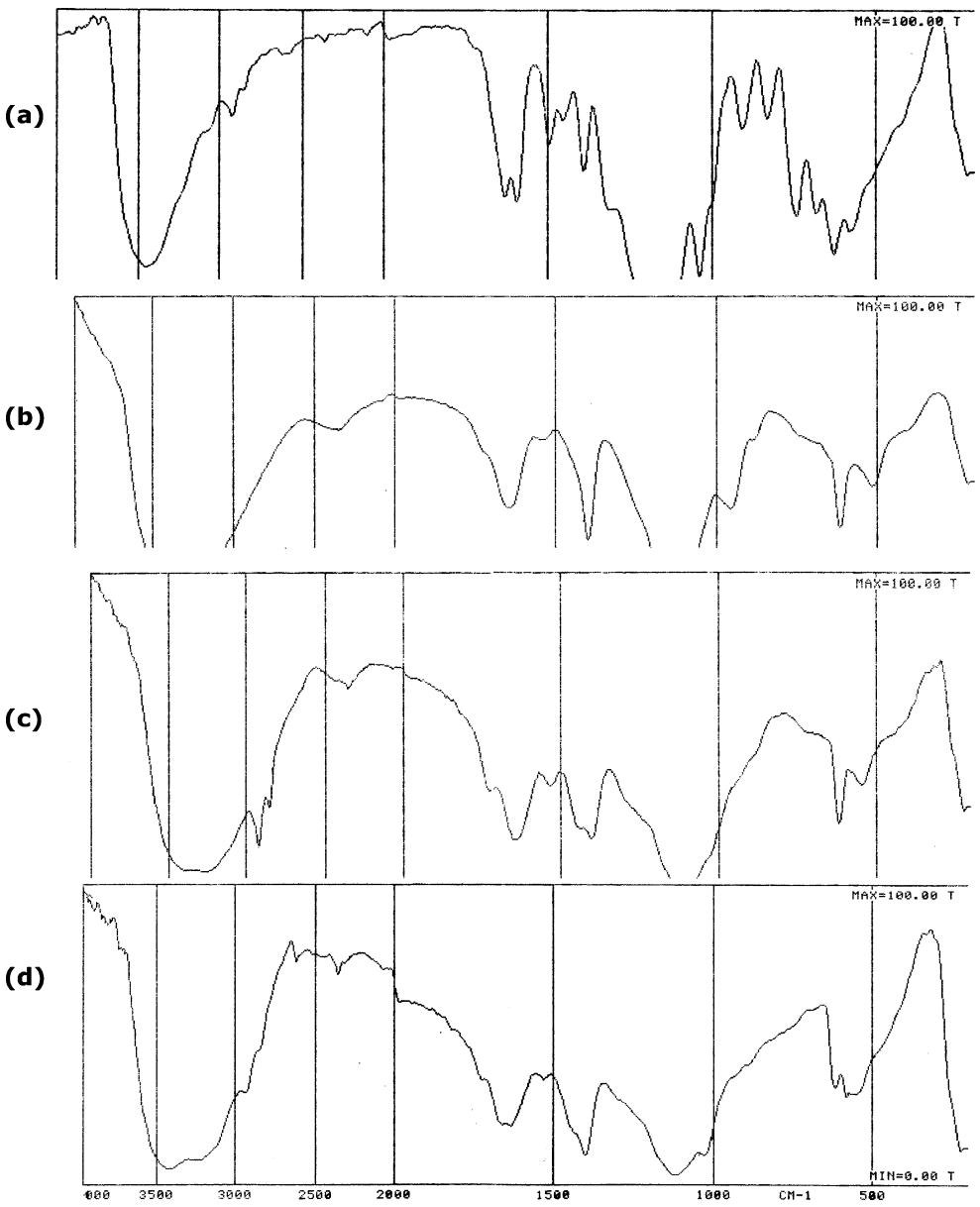

Fig. 4a–d Infrared (IR) spectra of Reactive Blue 21. **a** Control, **b** Reactive Blue 21 treated with *Penicillium lanosum*, **c** Reactive Blue 21 treated with *Fusarium oxysporum*, **d** Reactive Blue 21 treated with *Ganoderma resinaceum*

It seemed that color removal and possible mechanism of decolorization involved initial mycelial biosorption and subsequent biodegradation.

Dye removal by a fungus occurs either through bioaccumulation or biodegradation. The color of the fungal biomass after dye removal is an indicator of the principal mechanism behind dye removal. If the color of the fungal biomass takes on the color of the dye then the process is biosorption or bioaccumulation (Chen et al. 2003). On the other hand, using fluorescence microscopy, Roncero and Duran (1985) found a change in cell wall morphology and formation of rudimentary wall material in the case of *Geotrichum lactis* that was promoted by calcofluor white (fluorochrome dye).

IR analysis

As shown in Fig. 4, treatment of reactive Blue 21 dye with *P. lanosum* gave a band at υ 3,203 cm^{-1} that corresponds to the NH_2 group and a band at υ 3,382 cm^{-1} that corresponds to the NH group that is not present in the spectrum of the original dye. In the case of treatment of reactive Blue 21 dye with *F. oxysporum*, a band appears at υ 3,262 cm^{-1} corresponding to the NH_2 group that is not present in the spectrum of the original dye; however, a band at υ 1,111 cm^{-1} found as υ 1,039 cm^{-1} in the original dye corresponds to the C–O–C (ester) group, while a band at υ 1,595 cm^{-1} that corresponds to the C = C group disappears (bands at υ 1,533 cm^{-1} and υ 1,650 cm^{-1} refer to C = C gp and C = O groups, respectively).

Treatment of reactive Blue 21 dye with *G. resinaceum* gave a band at υ 3,423 cm^{-1}, that corresponds to the NH group and another at υ 1,595 cm^{-1} that was shifted to υ 1,532 cm^{-1} which corresponds to C = C groups. All changes in υ of the original dye of reactive Blue 21 tested with *P. Lanosum*, *F. oxysporum* and *G. resinaceum* indicated the ability of all three fungi to absorb the original dye.

Husseiny (2008) found the presence of an IR band at υ 3,364 cm^{-1} and a band at υ 3,534.8 cm^{-1} referring to a free NH_2 group that is not present in the spectrum of reactive Red 120 treated with *Penicillium* spp. Cao et al. (2001) and Stolz (2001) classified fungal decolorization of dye into two kinds according to their life state: living cells that biodegrade and biosorb dyes, and dead cells (fungal biomass) that adsorb dyes.

Conclusion

The current study indicates efficient dye biodegradation by the five tested fungal isolates (*P. lanosum*, *F. oxysporum*, *G. resinaceum*, *A. fumigatus* and *P. chrysogenum*). *P. lanosum*, *G. resinaceum* and *F. oxysporum* were the most efficient fungi in biodegrading the reactive Blue 21 dye under optimum conditions.

References

Abou-Okeil A (2005) Modified sawdust for dye removal. 2nd International Conference of Textile Research Division, NRC, Cairo, Egypt, April, 11–13.

Ali H, Muhammad SK (2008) Biodecolorization of acid violet 19 by *Alternaria solani*. Afr J Biotechnol 7(6):831–833

Ali H, Ahmad W, Haq T (2009) Decolorization and degradation of malachite green by *Aspergillus flavus* and *Alternaria solani*. Afr J Biotechnol 8(8):1574–1576

An SY, Min SK, Cha JH, Choi YL, Cho YS, Kim CH, Lee YC (2002) Decolorization of triphenylmethane and ago dyes by *Citrobacter* sp. Biotechnol Lett 24:1037–1040

Assadi MM, Jahangiri MR (2001) Textile waste water treatment by *Aspergillus niger*. Desalination 141:1–6

Basibuyuk M, Forster CG (1997) The use of sequential anaerobic/aerobic process for the biotreatment of simulated-dyeing waste water. Environ Technol 18(8):843–848

Bhatti HN, Akram N, Asgher M (2008) Optimization of culture conditions for enhanced decolorization of cibacron red FN-2BL by *Schizophyllum commune* IB1.6. Appl Biochem Biotechnol 149:255–264

Cao H, Hardin IR, Akin DE (2001) Optimization of conditions for microbial de colorization of textile waste water: starch as a carbon source. AATCC Rev 1:37–42

Chen KC, Wu JY, Liou DJ, Hwang SCJ (2003) Decolorization of the textile dyes by newly isolated bacterial strains. J Biotechnol 101:57–68

Churchley JH (1994) Removal of dye waste color from sewage effluent the use of a scale ozone plant. Water Sci Technol 30:275–284

Fu Y, Viraraghavan T (2001) Fungal decolorization of dye wastewaters: a review. Bioresour Technol 79:251–262

Govindaswami M, Schmidt TM, White DC, Loper JC (1993) Phylogenetic analysis of a bacterial aerobic degrader of azo dyes. J Bacteriol 175:6062–6066

Hartman CP, Fulk GE, Andrews AW (1978) Azo reduction of trypan Blue to known carcinogen by a cell-free extract of a human intestinal anaerobe. Mutat Res 58:125–132

Husseiny ShM (2008) Biodegradation of reactive and direct dyes using Egyptian isolates. J Appl Sci Res 4(6):599–606

Kapdan IK, Kargi F (2002) Biological decolorization of textile dyestuff containing wastewater by *Coriolus versicolor* in a rotating biological contactor. Enzyme Microb Technol 30:195–199

Kapdan IK, Kargi F, Mc MG, Marchan TR (2000) Effect of environmental condition on biological decolorization of textile dyestuff by *Coriolus versicolor*. Enzyme Microb Technol 26:381–387

Kim HT, Lee Y, Yan J, Lee B, Parch CH, Kim S (2004) Decolorization of dye solutions by a membrane bioreactor (MBR) using white rot fungi. Desalination 168:287–293

Lele SS, Madhavi S, Revankar L (2007) Synthetic dye decolorization by white rot fungus *Ganoderma* sp. WR.1. Bioresource Technol 98(4):775–780

Moubasher AH (1993) Soil fungi in Qatar and other Arab Countries University of Qatar, 1st edn. Center for Scientific and Applied Research, Qatar

Mustafa AA (2002) Studies on spawn and substrate problems for mushroom cultivation with special reference to some enzyme patterns. PhD, Botany Department Tanta University

Nigam P, Banat IM, Singh D, Marchant R (1996) Microbial process for the decolorization of textile effluent containing ago dye, diazo and reactive dyes. Process Biochem 31:435–442

Panswad T, Luangdilok W (2000) Decolorization of reactive dyes with different (environmental) molecular structures under different environmental conditions. Water Res 34:4177–4184

Rafii F, Freankalin W, Cernigilia CE (1990) Azo-reductase activity of anaerobic bacteria isolated from human intestinal microflora. Appl Environ Microbiol 56:2146–2151

Robinson T, Mcmullan G, Marchant R, Nigam P (2001) Remediation of dyes intextile effluents: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77:225–227

Roncero C, Duran A (1985) Effect of Calcofluor white and Congo red on fungal cell wall morphogenesis: in vivo activation of chitin polymerization. J Bacteriol 163:1180–1185

Santhy K, Selvapathy D (2006) Removal of reactive dyes from waste water by adsorption on coir pith activated carbon. Bioresour Technol 97:1329–1336

Shoda M, Lee TH, Aoki H, Sugano Y (2000) Morasses on the production and activity dye decolorizing peroxidase from *Geotrichum candidum*. J Biosci Bioeng 89(6):545–549

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microb Biotechnol 56:69–80

Tak HK, Lee Y, Yang J, Lee B, Park C, Kim S (2004) Decolorization of dye solutions by a membrane bioreactor (MBR) using white-rot fungi. Desalination 168:287–293

Toh Y, Jia J, Yen L, Obbard JP, Ting Y (2003) Decolorization of azo dyes by white-rot fungi (WRF) isolated in Singapore. Enzyme Microb Technol 33:569–575

Van Walbeek W, Scott PM, Thatcher FS (1968) Mycotoxins from food-borne fungi. J Microbiol 14:131–137

Yang Q, Yang M, Pritsch K, Yelidter A, Hagn A, Schloter M, Kettrup A, Schloter M, Kettrup A (2003) Decolorization of synthetic dyes and production of manganese-dependent peroxidase by new fungal isolates. Biotechnol Lett 25:709–713

Zheng Z, Levin RE, Pinkham JL, Shetly K (1999) Decolorization of polymeric dyes by a novel *Penicillium* isolate. Process Biochem 34:31–37

Zollinger H (1987) Color chemistry syntheses, properties and applications of organic dyes and pigments. VCH, New York