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Study on laboratory reactors for biodegradation of textile dyes by immobilized white-rot fungi mycelium

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A laboratory scale trickle-bed reactor (length of 20 cm, 7 cm i.d., bed volume of 385 cm^3) was constructed for the biodegradation of the Remazol Briliant Blue R and Reactive Orange 16 dyes by immobilized *Irpex lacteus* mycelium. Three carriers (Filtren TM30 polyether foam, polyamide kitchen scourers and lufa sponge slices) were chosen for the mycelium immobilization. Each carrier bed was characterized by the liquid hold-up value and the mean retention time at four volumetric flow rates of the liquid phase. Distributions of the retention times were also measured for each mycelium carrier at various liquid media flow rates. The lowest liquid hold-up value was achieved in the reactor packed with the plastic kitchen scourers, the largest value was observed in the reactor filled with the lufa sponge. The longest mean retention time, 430 s, was achieved in the reactor with the lufa carrier at liquid flow rate of 6.81 cm^3min^{-1}, the shortest mean retention time was achieved in the reactor filled with the plastic kitchen scourers.

Using various Filtren TM carriers any dependence of the activity of the enzymes laccase, lignin peroxidase and mangan peroxidase secreted by the mycelium of the fungus *Irpex lacteus* on the porosity of the carrier was not observed. The ability of *Irpex lacteus* to secrete laccase and mangan peroxidase enzymes in the bioreactor with all kinds of carriers was proved.

The ability of *Irpex lacteus* to biodegrade the dyes Remazol Brilliant Blue R and Reactive Orange 16 was verified experimentally. The degree of decolorization of 80% was achieved within 2 days in all reactors. In the experiments with the reactor packed with the plastic kitchen scourers and the lufa sponge slices the decolorization degree equaled 90% within 2 days.

1. Introduction

Long lasting focus on degradation of toxic substances in waste waters from the textile industry was even increased in recent years due to steadily growing environmental pollution. The pollutants from the textile industry are, in general, only hardly removable by traditional chemical and microbiological technologies devised for the municipal wastewater treatment. Due to their enzymatic systems, white-rot fungi mycelia immobilized on solid carriers are capable to biodegrade these residues (Cripps et al., 1990; Forgacs et al., 2004; Swamy and Ramsay, 1999; Ruiz-Aguilar et al., 2002; Novotný et al., 2001; Tavčar et al., 2006; Wang and Yu, 1998). *Irpex lacteus, Phanerochaete chrysosporium, Trametes versicolor* and *Pleurotus ostreatus* belong to the most often used fungi in the waste water treatment (Novotný et al., 2001; Tien and Kirk, 1984; Wesenberg et al., 2003). Most of the published literature concerning the

bioremediation of dyes focus on biological and biochemical aspects of the biodegradation phenomena, but only quite a few papers concern the construction of reactors (bio-filters) suitable for an application of the white-rot fungi mycelium. Stirred batch reactors or shaken flasks (see, e.g., Cripps et al., 1990; Novotný et al., 2001; Ruiz-Aguilar et al., 2002; Valentín et al., 2007), trickle bed reactors (Chang and Lu, 2004; Lu and Gang, 2004; Mielgo et al., 2001; Pielech-Przybylska et al., 2006) and rotating biological contactors (RBC reactor, see, e.g., Zheng and Obbard, 2002) represent the most frequently used reactor setups for the biodegradation processes performed by the white-rot fungi. Simple construction, an absence of moving parts and easy operation are the main reasons why the trickle bed reactor with the white-rot fungi immobilized on a suitable solid carrier is very often used in environmental applications (Chang and Lu, 2004; Lu and Chang, 2004; Mielgo et al., 2001; Pielech-Przbylska et al., 2006). Wood chips, polymer foams, ceramics particles, fibrous structures and special plastics biomass supporting particles can be used as the mycelium carriers. The most serious problem when these carriers and their beds are used represents their susceptibility to clogging by the growing mycelium.

The biodegradation of remazol brilliant blue R and reactive orange 16 dyes by the white-rot fungus *Irpex lacteus* immobilized on polyurethane foam in a small laboratory trickle bed reactors and in a rotating-disc reactor was studied recently by Tavčar et al., 2006 who confirmed applicability of the fungus for the wastewater treatment. The

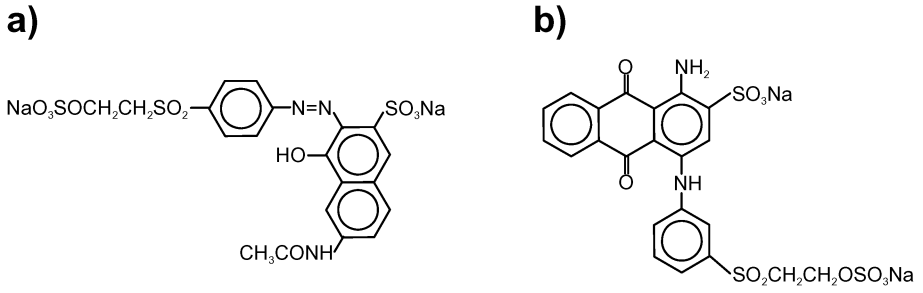

Figure 1: Structures of: a) *Remazol Brilliant Blue R* (*RBBR*), *and* b) *Reactive Orange 16* (*RO16*) *dyes*.

authors considered disc reactors as more suitable for the long term operation, in spite of the fact that their efficiency is slightly lower, as they prevent clogging by the mycelium biomas. The biodegradation of remazol brilliant blue R and reactive orange 16 dyes by the white-rot fungi *Irpex lacteus* immobilized on three macroporous carriers in a small laboratory trickle bed reactor was recently studied also by Pocedič, 2007 who proved practical applicability of all carriers used in his experiments.

Contrary to the biochemical and biological aspects of the bioremediation processes relatively low attention is devoted to studies on their engineering aspects, e.g., structure of the beds, the liquid phase hold-up, the wetting efficiency, residence time distributions, the axial dispersion etc. An attempt to fill some gaps in the knowledge of engineering parameters of the bioremediation processes and their relation to the biological efficiency of pollutants removal is done in this paper.

2. Materials and methods

2.1 Organism

The fungus *Irpex lacteus*, strain Fr. 238 617/93 (isolated from woods of the Czech Republic) was obtained from the Culture Collection of Basidiomycetes (CCBAS) of the Academy of Sciences of the Czech Republic, Prague.

2.2 Chemicals

The chemical structures of dyes used in decolorization experiments, namely remazol brilliant blue R (RBBR) and reactive orange 16 (RO16), are shown in Fig. 1 and were purchased from Sigma-Aldrich. All other chemicals used in experiments were of analytical grade and were purchased from local sources.

2.3 Bioreactor and mycelium carriers

The bioreactor was constructed of glass tube (20 cm long, 7 cm i. d., total bed volume 385 cm^3) sealed with two stoppers fabricated from the polydimethylsiloxane elastomer (Sylgard, DuPont, USA). The stoppers were equipped with tubular ports for the liquid and air inlets and outlets. A simple liquid distributor formed of a bunch of 5 curved Teflon capillaries was fitted to the upper stopper of the reactor. The distributor provided fairly even liquid distribution across the carrier beds at the liquid volumetric flow rates ranging from 5 to 25 cm^3min^{-1}. The overall bioreactor configuration is depicted in Fig. 2.

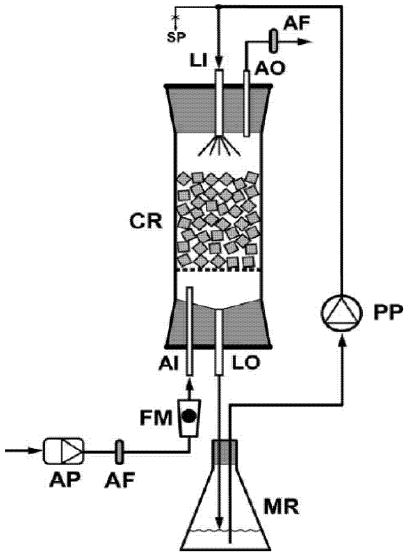

Figure 2: Schematics of the bioreactor and experimental set-up used for biodegradation experiments with immobilized mycelium of I. lacteus. CR… reactor tube; LI, LO … liquid inlet and outlet; AI, AO ... air inlet and outlet; FM … flow-meter; AF ... air filters; AP … air pump; MR … medium reservoir; PP … peristaltic pump; SP … sampling port.

Three kinds of mycelium carriers were used to evaluate their effects on the biodegradation of the RBBR and RO16 dyes: i) polyether macroreticulate foam Filtren® TM30 (Eurofoam, Brno, CZ) with inner porosity about 0.91, ii) the slices (thickness: 15 mm, diameter: 70 mm) of the cosmetic lufa sponge (dried pulp of *Luffa acutangula* fruits), and iii) polyamide kitchen scourers (height: 30 mm, diameter: 70 mm). Each carrier was three times washed in boiling distilled water prior its use. Configurations of carrier beds in the bioreactor are shown in Fig. 3.

2.4 Analytical methods

Decolorization

The degree of decolorization of liquid media was evaluated by the absorbance measurements at 592 nm for RBBR and at 490 nm for RO16.

Adsorption of the dyes to carriers used for immobilization
30 cm^3 of the aqueous dye solution (concentration: 150 mg dm^{-3}) was added to 1 g of dry carrier material cut to small pieces and the mixture was intensively shaken. The absorbance of the supernatants was measured at appropriate time intervals.

Liquid hold-up
The liquid medium was pumped to the reactor packed with the dry carrier bed at a constant volumetric flow rate. Amounts of the medium at both the inlet and the outlet of the reactor were recorded till steady values were reached. By material balancing the liquid hold-up in the carrier bed was evaluated.

Residence time distribution (*RTD*)
RTD of the liquid phase in the bioreactor was measured by standard stimulus–response method at four volumetric flow rates (6.8, 13.2, 19.6 and 25.2 cm^3min^{-1}). Copper(II) phthalocyanine-tetrasulfonic acid tetrasodium salt was used as the tracer and its concentration at the bioreactor outlet was measured at 694 nm by the spectrophotometer.

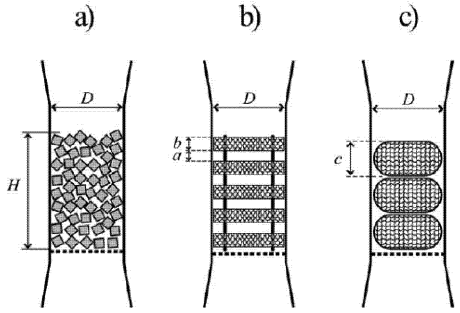

Figure 3: Configurations of mycelium carriers in the bioreactor. a) Filtren TM cubes (cube size 1×1×1 cm); b) lufa sponge slices; c) plastic kitchen scourers. Dimensions: D = 70 mm, H = 100 mm, a = 10 mm, b = 15 mm, c = 30 mm; total bed volume in bioreactor: approx. 385 cm^3.

2.5 Biodegradation experiments
The microorganism was first pre-cultivated on the agar plates (5 g dm^{-3} yeast extract, 10 g dm^{-3} nutrient agar, 10 g dm^{-3} glucose). The Erlenmeyer's flasks with 30 cm^3 of the Kirk's medium (Pocedič, 2007) were inoculated with the mycelium scraped from the agar plates after one week of the pre-cultivation. The *Irpex lacteus* mycelium was cultivated in the stationary flasks for one week at 28°C. Then the culture was homogenized by the Ultra-Turrax T25 homogenizator and the homogenate was used for the inoculation of the carriers. Each carrier together with the Kirk's medium was sterilized (121°C, 20 min) before addition of the homogenate. The Filtren TM30 carrier was inoculated in Erlenmeyer's flasks (total volume of 500 cm^3) filled with 30 cm^3 of fresh Kirk's medium to what 5 cm^3 of the mycelium homogenate was added. Plastics scourers were inoculated in separate bottles with 50 cm^3 of the Kirk's medium. 3 cm^3 of the homogenate was added to each piece of the scourers. Each lufa sponge slice was inoculated in the glass Petri dish with 30 cm^3 of the Kirk's media and 2 cm^3 of the homogenate was used per each slice. Inoculated carriers were then transferred to the bioreactor under sterile conditions and the decolorization experiments were started by pumping the medium. The volumetric flow rate of the medium was set to 20 cm^3min^{-1}.

The temperature was kept at 28°C during all decolorization experiments. The growth of the mycelium on the carriers was observed visually and the concentrations of the dyes in the liquid medium were measured at appropriate time intervals.

3. Results and Discussion

3.1 Liquid hold-up in carrier beds

Values of the liquid phase hold-up (expressed as the volumetric fraction of the liquid in the bed, ε_L) are listed in Table 1. The lowest values of the hold-up were obtained with the plastic kitchen scourers as the scourers material (a polyamide) exhibits very poor surface wettability and is totally non-porous. This material would thus be, in principle, unsuitable as the carrier in the bio-filters. The Filtren TM30 polyether foam with the cellular structure is very suitable for mycelium immobilization and the liquid phase hold-up values in this carrier were quite high. The highest hold-up values were obtained with the lufa sponge carrier due to its highly porous structure and high surface wettability. The lufa sponge, however, exhibited large volumetric instability of the bed. The liquid hold-up values in all studied beds exhibit rather irregular dependence on the liquid phase volumetric flow rate, probably due to experimental errors.

Table 1: Liquid hold-up in the carrier beds

Liquid flow rate [cm^3min^{-1}]	**Filtren TM30** ε_L [%]	**Plastic scourers** ε_L [%]	**Lufa sponge** ε_L [%]
6.81	1.99	0.29	1.93
13.23	1.48	0.93	3.93
19.59	1.84	1.39	3.43
25.18	1.62	0.79	2.77

3.2 Dyes adsorption to carriers

Results for both dyes and for all support media are summarized in Table 2. It is obvious that both tested dyes exhibited no adsorption to either of the tested carriers. Therefore the rate of dyes biodegradation is not influenced by the adsorption processes.

3.3 RTD of liquid phase

The experimentally determined residence time distribution (RTD) functions in the biorector packed with all three carriers at fixed liquid volumetric flow rate value of 6.81 cm^3min^{-1} are shown in Fig. 4. The RTDs for the bioreactor packed with the Filtren TM30 cubes and with the plastic kitchen scourers share common features: The residence times are relatively short as the liquid hold-up within the bed is low. The liquid phase flow in the bioreactor can be described by a model of the axial dispersion combined with the dead zones within the bed resulting to apparent skewness of the RTDs towards the longer residence times. The residence times in the bioreactor packed with the lufa sponge slices are significantly longer due to the diffusion of the tracer dye into the lufa sponge material (the Filtren foam and the scourers materials are impermeable for the tracer).

3.4 Biodegradation experiments

Two parallel biodegradation experiments with the column bioreactor for each of both dyes were performed – see Fig. 5. In the case of the RBBR dye 90% degree of

Table 2: Adsorption of dyes RBBR and RO16 on mycelium carriers

RBBR[1)]	**lufa sponge slices**	**plastic kitchen scourers**	**Filtren TM30 foam**
time [minutes]	average absorbance	average absorbance	average absorbance
0	0.669	0.669	0.657
10	0.673	0.665	0.664
30	0.659	0.672	0.664
120	0.671	0.676	0.666
1440	0.694	0.689	0.665
R016[2)]	**lufa sponge slices**	**plastic kitchen scourers**	**Filtren TM30 foam**
time	average absorbance	average absorbance	average absorbance
0	0.749	0.756	0.759
10	0.760	0.755	0.783
30	0.753	0.793	0.766
120	0.743	0.749	0.757
1440	0.738	0.770	0.740

[1)] 0.5021 g in 25 cm^3; [2)] 0.5223 g in 25 cm^3

decolorization was achieved in two days. The same rates of the biodegradation were observed for the lufa sponge and the plastic scourers carriers, the lower rate was observed with the Filtren TM30 carrier, probably due to limitation of the mycelium growth within the foam pores, where the oxygen and the substrate transport rates can be strongly limited. Some clogging problems due to the growing mycelium occurred in the Filtren cubes beds (Pocedič, 2007). The clogging does not pose a serious problem with the lufa sponge and the plastic scourers carriers. The biodegradation of the RO16 dye in the bioreactor proceeded in a quite similar way – cf. Fig. 5. The rate of the decrease of the RO16 concentration was, however, especially at the beginning stage of the experiment, notably lower and only 85% degree of the decolorization was observed after 4 days.

4. Conclusions

The decolorization experiments performed in a small scale laboratory trickle-bed bioreactor with three different kinds of solid carriers with the immobilized mycelium of *Irpex lacteus* Fr. 238 617/93 proved its ability to decolorize the dyes RBBR and RO16. The decolorization was achieved within 2–4 days). All carriers tested proved to be almost identically efficient in the decolorization experiments despite huge differences in their hydrodynamical properties. Fundamental process parameters characterizing the bioreactor were determined over a range of operating conditions of the bioreactor. The knowledge gained will be used in design of a larger scale bioreactor.

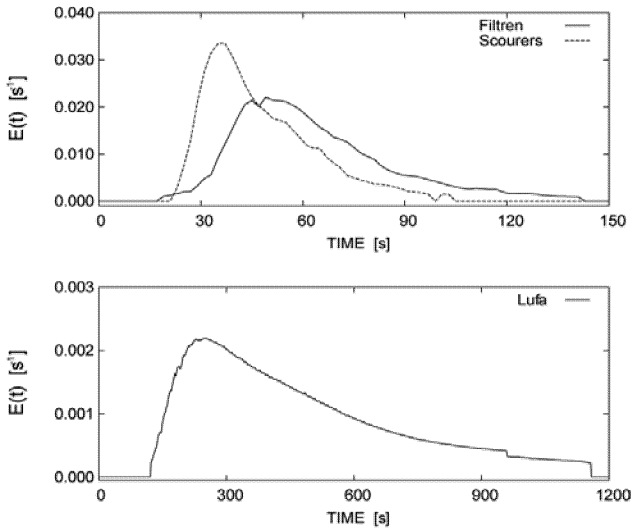

Figure 4: RTD of liquid phase in bioreactor packed with different carriers.

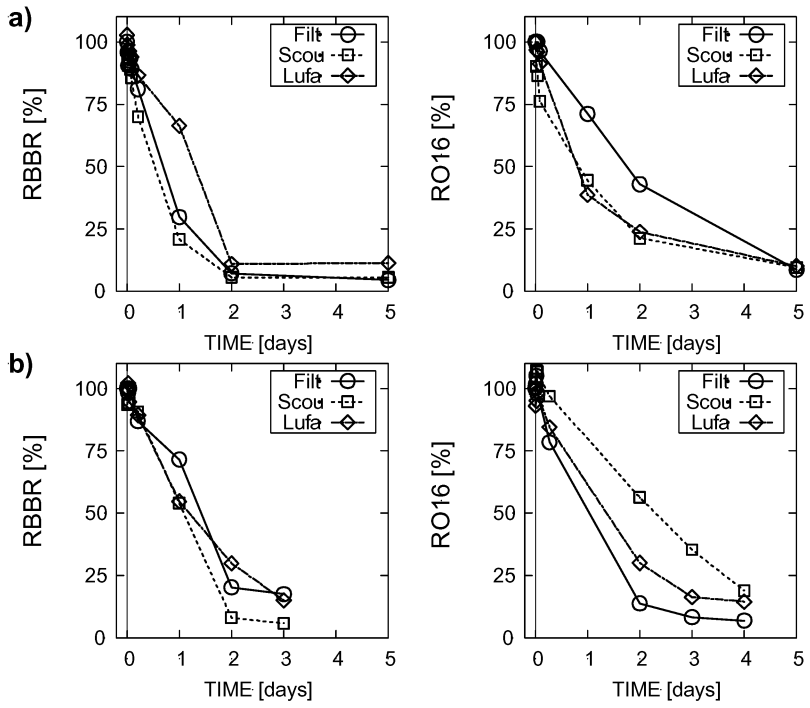

Figure 5: Time course of decolorizations of RBBR and RO16 dyes in two experiments (a, b). Concentrations of dyes are related to their initial values. Filt …Filtren TM30 carrier, Scou … plastic scourer carrier, Lufa …lufa sponge

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