

# Rotating biological contactor and its application for decolorization of textile dyes by *Irpex lacteus*

J. Pociedič, O. Knotek, J. Šíma, P. Hasal

Department of Chemical Engineering, Institute of Chemical Technology,  
Prague, Technická 5, 166 28 Praha 6, Czech Republic,  
E-mail: Jaromír.Pocedic@vscht.cz, Pavel.Hasal@vscht.cz

## Introduction

Residual dyes, salts and detergents in outlet streams from textile industries represent serious environmental problem. A common outlet stream from textile industry typically contains from 10 to 200 mg/L of the dye. Composition of the outlet stream can vary substantially during a process in correspondence to the actual step in the dyeing process (Doble and Kumar, 2005). When water with the residual dye is submitted to the classical waste water cleaning process, numerous problems may arise. For example, cleavage of the chromogenic groups does not proceed during the aerobic process, while a formation of carcinogenic or otherwise toxic substances may occur in the anaerobic process (Dua et al., 2002).

In recent years, the wood-decaying fungi (white-rot fungi) or their enzyme systems have been extensively used for decolorizations of the textile dyes (see, e.g., Kapdan and Kargi, 2002 and Hailei et al., 2009). The ability of the white-rot fungi to decolorize various toxic substances is due to biosynthesis of non-specific enzymes, especially laccases, lignin peroxidases and manganese peroxidases. These enzymes are able to cleave the bonds in the lignin macromolecules and allow the white-rot fungi to utilize it as the carbon source and also enable to oxidize a number of organic substances (Knapp et al., 1995; Swamy a Ramsay, 1999). The white-rot fungi species most frequently used for removal of organic compounds from waters are *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus* and *Irpex lacteus*.

The laccase of the wood-decaying fungus *Irpex lacteus*, contrary to the other ones listed above, remains bound to the external surface of the mycelium and this fungus therefore appears as a proper candidate for the use in reactors with the growing mycelium (Svobodová et al., 2007). However, white-rot fungi mycelia are quite fragile and prone to shear stress damage when used in liquid cultures. For this reason it is predominantly used in an immobilized form using a suitable solid carrier.

Most decolorization experiments with white rot fungi were performed in static cultures or shaken flasks and only scarce attempts have been done to use the fungi in larger scale reactors. Reactors most commonly used for decolorization and similar purposes are: trickle-bed reactors (e.g., Novotný et al, 2001), rotating biological contactors (e.g., Tavčar et al., 2006), fixed bed reactors (e.g., Feijoo et al., 1994) and membrane reactors (e.g., Katuri et al., 2009). Rotating biological contactor (RBC) represents one possible reactor configurations for applications of the wood-decaying fungi due to its favourable hydrodynamic conditions and possibility of treating highly polluted waters. RBC typically consists of a set of rotating discs mounted to the common shaft placed in a reservoir. Polymer foams, metal sponges, wood or other natural materials are used as

the material for the discs fabrication. The advantage of usage of porous materials is to allow better attachment of the mycelium, while the advantage of the usage of natural materials is the enhanced induction of enzyme biosynthesis and thereby the increase of the speed of decolorization. The ability of white-rot fungus *Irpex lacteus* decolorize the dye Reactive Orange 16 in a rotating disc reactor with discs made from a macroporous foam were studied by Tavčar et al, 2006. One of the most important parameters affecting the decolorization rate in the RBC is the speed of rotation of the discs. In this paper we focus on design of a laboratory-scale rotating disc biofilter and verification of a capability of the wood-decaying fungus *I. lacteus* decolorize the textile dyes Remazol Brilliant Blue R and Reactive Orange 16 using this reactor under various conditions.

## Material and methods

### Organism

Wood-decaying fungus *Irpex lacteus* (strain Fr. 238 617/93 isolated from the forests of the Czech Republic) was obtained from the Culture Collection of Basidiomycetes of the Academy of Sciences of the Czech Republic (CCBA).

### Chemicals

All chemicals used were of the analytical quality and were purchased from local sources. Two chemically different dyes were used: the anthraquinone dye Remazol Brilliant Blue R (RBBR, Sigma-Aldrich) and the azodye Reactive Orange 16 (RO16, Sigma-Aldrich).

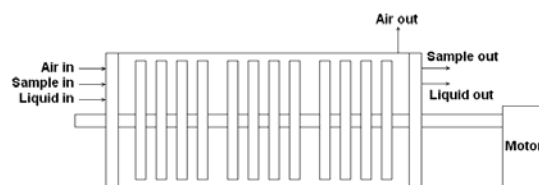


Fig. 1: Schematics of the rotating discs biofilter (side view). The length of the reservoir is 300 mm and its cross section is 150 × 150 mm (with rounded bottom). Stepper motor controlled by PC is used for discs rotation.

### Growth media

Solid agar medium for storage of the mycelium was composed of 20 g nutrient agar, 5 g malt extract and 10 g glucose per 1 L of the medium.

Liquid Kirk's medium with low nitrogen content (pH 4.5) containing 0.1 g L<sup>-1</sup> of ammonium tartrate as the nitrogen source was sterilized at 121°C for 20 min and used for the cultivation of mycelia and for decolorization experiments (Pocedič et al., 2009). Sterilized solutions of the dyes in distilled water were added to the Kirk's medium in order to achieve required initial concentration of the dye in the solution.

### Rotating biological contactor

Laboratory scale rotating biological contactor (RBC) used in this work (see Figs. 1 and 2) was constructed of polycarbonate plates forming the body (a reservoir) of the reactor. The reactor is equipped with stainless steel axis with 12 discs (diameter: 13 cm, thickness: 1 cm) serving as carriers for the mycelium. Filtren TM reticulated polyether foam (Eurofoam TP, Czech Republic) and the pine wood were used for fabrication of

the discs. The discs were intensively washed three times with boiling distilled water before use to remove possible soluble residues. The reactor was equipped with necessary inlet and outlet ports for liquid and gaseous streams and with the sampling ports. The entire reactor was sterilized by autoclaving at 121°C for 20 minutes prior inoculation. The microorganism was inoculated on each disc using 2 mL of the homogenized *I. lacteus* mycelium, prepared as follows: Three targets of the fungus grown on the solid agar medium in Petri dishes were aseptically transferred to 50 mL of the Kirk's medium in Erlenmeyer flasks and after 7 days of cultivation at 28 °C the contents of the flasks was homogenized by the ULTRA TURAX T18 homogenizer. The discs were mounted into the reactor and the reservoir was filled with 1500 mL of the Kirk's medium, and the stepper motor was turned on at 3 RPMs. The mycelium was allowed to grow for 14 days to colonize the discs. Then the reactor was ready for decolorization experiments. Operating temperature of the reactor was kept at 28 °C in all experiments. The experiments were started by filling the reactor with 1500 mL of the dye solution in the Kirk's medium and starting rotation of the discs at the chosen speed.

### **Dye concentration assay**

The concentrations of the dyes in decolorization experiments were determined by measuring absorbance of withdrawn samples at 490 nm for RO16 and at 592 nm for RBBR.

### **Laccase activity assay**

Time profiles of the laccase activity were determined in chosen decolorization experiments. 100 µL of the medium sample taken from the reactor and 355 µL of sodium tartrate buffer were mixed in a cuvette and the reaction was started by adding 100 µL of the ABTS solution and absorbance of the mixture at 420 nm was measured (ABTS = 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). Activity of the enzyme is expressed in  $U L^{-1}$ , where 1 U is the amount of the enzyme that creates 1 micromole of the product within 1 minute.



Fig. 2: Rotating disk biofilter with the *Irpex lacteus* mycelium attached to the Filtren TM discs.

## **Results and discussion**

### **Effects of the discs material on decolorization process**

The nature and physico-chemical properties of the material used for the discs fabrication may affect both the rate of the decolorization as well as the amount of secreted enzymes in the RBC. Two materials were chosen in this work: pine wood as a representative of the natural material and the Filtren TM30 foam as a neutral synthetic material.

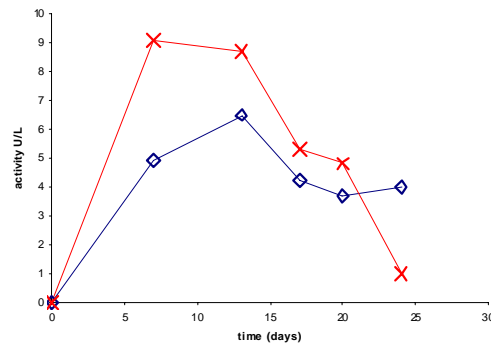


Fig. 3: Laccase activity during decolorization experiments; blue diamonds – Filtren TM discs, red crosses – wooden discs.

Comparing the average amounts of laccase secreted during decolorization experiments (see Fig. 3), we see that the enzyme activity in the reactor with the wooden discs was always higher throughout the experiment except the last day. Here, probably, began to dominate an impact of the abundant growth of the biomass on the wooden discs, which could begin to limit the excretion of the enzyme to the medium via strong mass transfer resistances. Higher laccase activity with the wooden discs was due to the presence of the lignin that positively affects the amount of the excreted enzyme, which corresponds with the results obtained by Šušla et al., 2008.

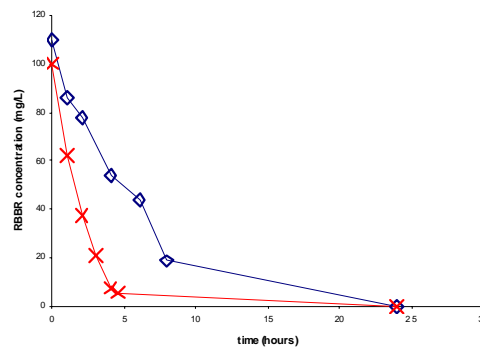


Fig. 4: Decolorization experiment: RBBR at initial concentration of  $100 \text{ mg L}^{-1}$ ; blue diamonds - Filtren TM discs, red crosses – wooden discs.

Looking at the dependence of the concentration of the dye on time in decolorization experiment (initial RBBR concentration  $100 \text{ mg L}^{-1}$ ) shown in Fig. 4, we see that the rate of decolourization in the reactor with the wooden discs is considerably higher than in the reactor with the Filtren TM discs. This finding correlates with the amount of the laccase secreted to the liquid media.

#### Effects of speed of discs rotation on dyes decolorization

The speed of rotation of the discs in the RBC represents one of the fundamental parameters that may affect the rate of the decolorization. The higher speed of rotation leads to substantial intensification of liquid phase mixing and  $l-s$  mass transfer rate, but it also leads to higher shear-stressing of the mycelium attached to discs. An experiment was conducted in order to elucidate impacts of change of rotation speed from

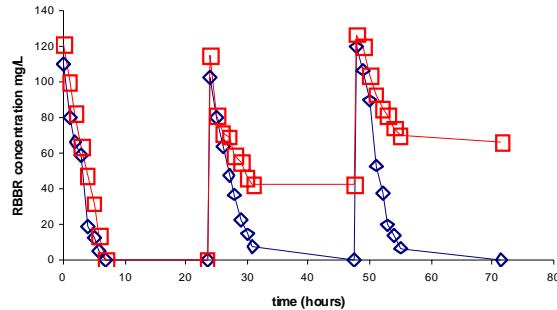


Fig. 5: Effects of the speed of discs rotation on RBBR decolorization in three consecutive experiments in RBC; blue diamonds - 5 RPM, red squares - 25 RPM.

5 RPMs to 25 RPMs on the decolorization process. The increase in the speed of rotation had clearly negative effects on the decolorization (see Fig. 5). In three consecutive decolorization experiments huge reduction in both the rate and the maximum decolorization was observed. Kapdan and Kargo, 2002, however, used white-rot fungus *Coriolus versicolor* in their RBC and observed an increase of efficiency of decolorization with the increasing speed of rotation. This difference may be due to fact that in case of the white-rot fungus *Irpex lacteus* the laccase is bound to the surface of the mycelium and higher speeds of rotation may lead to laccase leaching from the mycelium and to its inactivation.

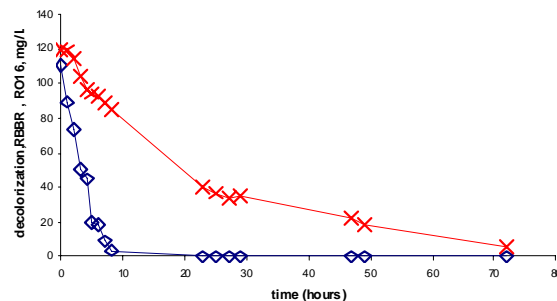


Fig. 6: Comparison of RBBR and RO16 decolorization rates in RBC; initial dyes concentration: 100 mg L<sup>-1</sup>, 5 RPM; blue diamonds - RBBR, red crosses - RO16.

### Comparison of RBBR and RO16 decolorizations

From comparison of the decolorizations of two chemically different dyes, RBBR and RO16, shown in Fig. 6 we can clearly observe markedly different decolorization rates. The 95% of the RBBR (the anthraquinone dye) is decolorized within 8 hours, whilst the same decolorization of the RO16 (the azo dye) is achieved only after 70 hours.

## Conclusions

The results presented in this paper demonstrate that application of the rotating disc biofilter with the disc-immobilized mycelium of the white-rot fungus *Irpex lacteus* represents favourable option for decolorization of waste waters containing textile dyes. When the Filtren TM foam is used as the immobilization support, the reactor is capable to remove effectively the Remazol Brilliant Blue R dye within few hours, while the Reactive Orange 16 requires a few days for the same degree of decolorization. When the softwood is used as the support material for the mycelium substantial increase of the decolorization speed is observed due to increased production of the laccase. The increase in the speed of rotation of the discs in the RBC has vastly negative effects on RBBR decolorization by *Irpex lacteus*.

**Acknowledgments:** This work was supported by the Ministry of Education of the Czech Republic (Grant: MSM6046137306), the Grant Agency of the Academy of Sciences of the Czech Republic (Grant: IAAX002200901) and the Grant Agency of the Czech Republic (Grant: 104/08/H055).

## References

- Doble M., Kumar A., 2005, Degradation of dyes, Elsevier - Butterworth Heinemann.
- Dua M., Singh A., Sethunathan N., Johri A. K., 2002, Biotechnology and bioremediation: successes and limitations, *Appl Microbiol Biotechnol* 59, 143-152.
- Feijoo G., Dosoretz C., Lema J. M., 1994, Production of lignin peroxidase from *Phanerochaete chrysosporium* in a packed bed bioreactor with recycling, *Biotechnol Tech* 8, 363-368.
- Hailei W., Ping L., Min P., Zhijun Z., Guangli Y., Guosheng L., Jianming Y., 2009, Rapid decolorization of azo dyes by a new isolated higher manganese peroxidase producer: *Phanerochaete* sp. HSD, *Biochem Eng J* 46, 327-333.
- Kapdan I. K., Kargi F., 2002, Biological decolorization of textile dyestuff containing waste water by *Coriolus versicolor* in a rotating biological contactor, *Enzyme Microb Technol* 30, 195-199.
- Katuri K. P., Mohan S. V., Sridhar S., Pati B. R., Sarma P. N., 2009, Laccase-membrane reactors for decolorization of an acid azo dye in aqueous phase: process optimization, *Water Res* 43, 3647-3658.
- Knapp J. S., Newby P. S., Reece L. P., 1995, Decolorization of dyes by wood-rotting basidiomycete fungi, *Enzyme Microb Technol* 17, 664-668.
- Novotný Č., Rawal B., Bhatt M., Patel M., Šašek V., Melitoris H. P., 2001, Capacity of *Irpex lacteus* and *Pleurotus ostreatus* for decolorization of chemically different dyes, *J Biotechnol* 89, 113-122.
- Pocedič J., Hasal P., Novotný Č., 2009, Decolorization of organic dyes by *Irpex lacteus* in a laboratory trickle-bed biofilter using various mycelium supports, *J Chem Technol Biotechnol* 84, 1031-1042.
- Svobodová K., Senholdt M., Novotný Č., Rehorek A., 2007, Mechanism of reactive orange 16 degradation with the white rot fungus *Irpex lacteus*, *Process Biochem* 42, 1279-1284.
- Swamy J., Ramsay J. A., 1999, The evolution of white rot fungi in the decoloration of textile dyes, *Enzyme Microb Technol* 24, 130-137.
- Šušla M., Novotný Č., Erbanová P., Svobodová K., 2008, Implication of *Dichomitus squalens* manganese-dependent peroxidase in dye decolorization and cooperation of the enzyme with laccase, *Folia Microbiol* 53, 479-485.
- Tavčar M., Svobodová K., Kuplenk J., Novotný Č., Pavko A., 2006, Biodegradation of azo dye RO16 in different reactors by immobilized *Irpex lacteus*, *Acta Chim Slov* 53, 338-343.