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Hydrodynamics of a Laboratory Scale Rotating Biological Contactor and its Application for Decolorization of Textile Dyes by White Rot Fungus *Irpex lacteus*

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Textile industries produce huge amounts of colored wastewaters contaminated with various textile dyes. These waters represent serious environmental problem as common wastewater treatment procedures are not efficient to degrade the dyes. Therefore, new ways of textile wastewater treatment are looked for in order to tackle this environmental problem. An application of the white rot fungus *Irpex lacteus*, that is known to degrade many recalcitrant pollutants, seems to be one of possible options for the waste water decolorization. A laboratory scale Rotating Biological Contactor was constructed to perform the decolorization tests. The reactor vas equipped with 12 flat circular discs (ø = 0.13 m, δ = 0.01 m) mounted on a common shaft. The discs were made of the Filtren TM30 reticulated polyether foam. Volume of the liquid in the reactor was 1.5 dm^3. Residence time distributions of the liquid phase in the reactor were measured to obtain hydrodynamic characteristics. The measurements were carried out at various liquid volumetric flow rates and at different rotational speeds of the discs uncovered with the biomass. The measured data were used to determine the liquid mean residence time and parameters of the gamma distribution model and of the model of the stirred tanks in series with the back-flow. The residence time distributions were also measured after 36 days of the reactor operation, i.e., with the discs covered with the *Irpex lacteus* mycelium biofilm. The flow behavior of the reactor without the fungus was close to the ideally stirred reactor behavior. The presence of the biomass in the reactor brought about deviations from the ideal mixing. Continuous decolorization experiments with the textile dye Remazol Brilliant Blue R and with a sample of an industrial textile waste water were performed. In the course of the experiments, activities of the enzymes involved in the dye decolorization, i.e., laccase, manganese-dependent peroxidase and lignin peroxidase were determined in the outlet stream of the reactor. The inlet and outlet concentrations of glucose were also measured. The results of the decolorization experiments will be presented and mutually compared and effects of hydrodynamic parameters on decolorization will be discussed.

1. Introduction

Recovery of waste waters from textile industries represents serious environmental problem. This waste water usually contains various residual dyes, salts, detergents and other agents. The composition of water can vary very quickly according to running processes in the plant. Unfortunately, common sewage treatment plants are not convenient to treat waste water from the textile industry as the textile dyes are highly resistant to common waste water treatment procedures.

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In recent years, white rot fungi have been extensively studied because of their ability to decolorize wide range of dyes (Novotný et al., 2001). This capability is often associated with production of ligninolytic enzymes as laccase, manganese-dependent peroxidase (MnP) and lignin peroxidase (LiP) (Novotný et al., 2004a). These enzymes are nonspecific and they can degrade a lot of chemical substances (Novotný et al., 2004a; Ollikka et al., 1993). The most frequently used fungi to remove dyes from a wastewater are *Trametes versicolor*, *Pleurotus ostreatus* and *Irpex lacteus*. In our work we focused especially to the last one, which was found to be effective in degradation of many synthetic textile dyes under various growth conditions (Novotný et al., 2004b).

Immobilized microorganisms are usually used in wastewater treatment as immobilization brings about some advantages such as continuous reactor operation without a risk of the cell washout, resistance of cells against the toxicity and environmental stresses and a high cell density. On the other hand, it can cause problems related to the diffusion, a change of the microbial population and sometimes immobilization can be expensive (Bitton, 2005). A trickle bed reactor (TBR) (Pocedič et al., 2009) and a rotating disc contactor (RDC) are very often used reactor configurations in the decolorization of the textile dyes by fungi. According to Tavčar et al. (2006) the TBR is slightly more efficient than the RDC, but the RDC is more resistant to clogging by abundantly growing fungus compared to the TBR. RDCs are reliable devices and have relatively low power consumption (Patwardhan, 2003). For this reasons we have chosen this type of reactor for our experiments.

A RDC usually consists of set of discs mounted on a common shaft and rotating in a vat. The discs can be made of various materials. The properties of the material may significantly affect the rate of the decolorization and the amount of enzymes secreted by the fungus. Pocedič et al. (2010) used the pine wood and a polyether foam (Filtren TM30) discs. With the wooden discs the activity of the laccase secreted by *Irpex lacteus* was remarkably higher than in the case of the polyether discs. Consequently, the rate of decolorization of the dye Remazol Brilliant Blue R (RBBR) was higher with wooden discs. The discs are usually immersed to about 40 % in the liquid, but in anaerobic processes the disc can be immersed deeper (Rodgers and Zhan, 2003). The rotational speed of the discs is the very important operational parameter of the RDC. It affects reactor hydrodynamics, concentration of dissolved oxygen in the liquid and the mass transport.

Besides the kinetics of the reaction it is also relevant to know the hydrodynamics of the liquid in the reactor. The flow behaviour of real reactors usually lies somewhere in between of two ideal cases: the ideal mixer and the plug-flow system. Various mathematical models are used to describe extent of the liquid backmixing in reactors (Buffham and Gibilaro, 1968). The parameters of the models are usually estimated from tracer experiments results. A frequently used mathematical model of flow systems is the model of a series of stirred tank reactors (Stokes and Nauman, 1970) that can describe a variety of flow regimes from a single well-stirred reactor to the system with the plug flow (Buffham and Gibilaro, 1968).

In this paper we first focus on design of a laboratory-scale rotating disc biofilter and on a study of its hydrodynamics under various operational conditions. Then we make an attempt to use this reactor equipped with the discs made of a polyether foam (Filtren TM30) to decolorize the RBBR dye and a real textile waste water.

2. Material and methods

2.1 Microorganism

Decolorization experiments were performed using the white-rot fungus *Irpex lacteus* (strain Fr. 238 617/93), which was isolated from the forests of the Czech Republic. This fungus was obtained from the Culture Collection of Basidiomycetes (CCBAS) of the Academy of Sciences of the Czech Republic.

2.2 Chemicals

Anthraquinone dye Remazol Brilliant Blue R (RBBR) was purchased from Sigma-Aldrich and real textile waste water containing Sumifick Black B (9.82 g dm^{-3}) and Inozin Yellow V-GR (2.46 g dm^{-3}) was obtained from Inotex s.r.o. (Dvůr Králové, Czech Republic). The tracer experiments without fungus were carried out with the azo dye Reactive Orange 16 (RO16, Sigma-Aldrich) as a tracer. The dye

Copper(II) phtalocyaninetetrasulfonic acid (CuP, Aldrich) served as the tracer in experiments after reactor inoculation. All other chemicals used in the experiments were gained from local sources and were of analytical grade.

2.3 Culture media

Fungus *Irpex lacteus* was stored on solid agar medium with 0.5% (W/V) malt extract, 1% (W/V) glucose and 2% (W/V) nutrient agar (Tavčar et al., 2006). For the decolorization experiments and for the fungus cultivation either the Kirk's medium with low content of nitrogen (0.1 g L^{-1}, Tien and Kirk, 1988) or the malt extract medium with 1% (W/V) glucose and 0.5% (W/V) malt extract was used. The pH value was kept at 4.5. The media were sterilized at 121°C for 20 min.

2.4 Rotating discs contactor

A laboratory-scale rotating discs contactor (RDC) was used in this study. The RDC consists of a body (round bottomed vat) and 12 discs (13 cm diameter, 1 cm thickness) mounted on a stainless steel shaft (see Figure 1). The inner reservoir dimensions were: 31.2 cm length, 15.6 cm height, 16.2 cm width (see Figure 1a), the filling volume of the liquid was 1.5 dm^3, total inner volume is 7×10^{-3} m^3. The discs were made of the macroporous reticulated polyether foam (Filtren TM30, Eurofoam TP, Czech Republic). The reactor was equipped with gas and liquid inlets and outlets (see Figure 1b) made of stainless steel capillaries. The shaft with the discs was rotated by a PC controlled stepper motor.

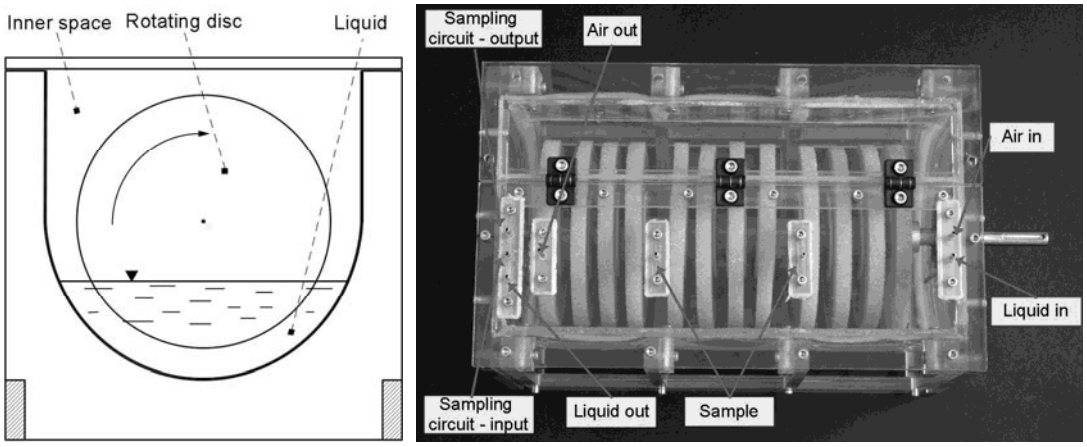

Figure 1: Rotating discs contactor (RDC): a) Cross sectional view; b) Locations of inlets and outlets

2.5 Reactor inoculation

Prior the inoculation the discs were thoroughly washed with the boiling distilled water to remove soluble residues and the entire RDC was sterilized in the autoclave at 121°C for 20 min. Each disc was inoculated with 5 mL of homogenized *Irpex lacteus* mycelium prepared as follows: Three circular targets (1 cm) of fungus grown on solid agar medium were aseptically moved from Petri dishes to Erlenmeyer flasks with 50 mL of Kirk's medium. After 7 days of static cultivation at 28 °C the contents of the flask was homogenized by ULTRA TURRAX T18 homogenizer and the homogenate was used for inoculation (cf. Pocedič et al., 2010).

2.6 Dye concentration assay

The concentrations of dyes were determined by measuring the absorbance of samples at 490 nm for RO16, 592 nm for RBBR, 600 nm for Sumifix Black B, 380 nm for Inozin Yellow V-GR and 690 nm for CuP.

2.7 Enzyme activity assays

The laccase activity was determined by ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay (Kunamneni et al., 2008), the manganese-dependent peroxidase (MnP) activity by DMAB/MBTH

(3-dimethylaminobenzoic acid/3-methyl-2-benzothiazoline hydrazone hydrochloride) assay and the lignin peroxidase (LiP) activity by veratryl alcohol assay (Vyas et al., 1994). The activities of the enzymes were measured at 28°C. One unit (U) of enzyme activity is defined as the amount of enzyme producing 1 μmol of a product per minute (MnP, laccase) or consuming 1 μmol of the substrate per minute (LiP).

3. Results and discussion

3.1 Hydrodynamic characterization of RDC

To characterize hydrodynamics of the reactor the residence time distribution was measured by step-response tracer experiments. The measured data was used to determine parameters of two flow models: the stirred tanks in series with back flow model (TSBF) and the model of gamma distribution (GDM). The TSBF model consists of the following *n* mass balances of the tracer:

$$\frac{\mathrm{d}c_1}{\mathrm{d}\theta} = n\left[c_{in} + b\,c_2 - (b+1)\,c_1\right] \quad , \tag{1}$$

$$\frac{\mathrm{d}c_k}{\mathrm{d}\theta} = n\left[(b+1)\,c_{k-1} - (b+2)\,c_k + b\,c_{k+1}\right] \quad , \quad i = 2,...,n-1, \, , \tag{2}$$

$$\frac{\mathrm{d}c_n}{\mathrm{d}\theta} = n\left[(b+1)\,c_{n-1} + (b-1)\,c_n\right], \; c_{in} = 1 \text{ for } \theta \geq 0 \text{ and } c_k = 0 \text{ at } \theta = 0. \tag{3}$$

The set of Eqs. (1) - (3) has to be solved numerically.

The gamma distribution model is described by the probability density function of residence times – the impulse response in the form (Buffham and Gibilaro, 1968):

$$f(\theta) = \frac{n^n\,\theta^{n-1} e^{-n\cdot\theta}}{\Gamma(n)} \cdot \tag{4}$$

The parameter *n*, corresponding to the number of stirred tanks in series, may take non integer values in this model. Residence time distributions were measured at various liquid flow rates *Q*, the discs rotated at 3 rpm (see Table 1). Values of model parameters were calculated using Mathematica 7 software (Wolfram Research) by minimization of sum of squares of deviations of calculated and measured residence time distributions.

The results in Table 1 and Figure 2 indicate that the flow behaviour of the RDC is close to the continuous stirred tank reactor (CSTR) behaviour at all flow rates. The parameter *n* of the model of stirred tanks in series with the back flow (TSBF) takes low values (2 or 3) and the back-flow parameter *b* takes, in general, quite high values. The values of the parameter *n* of the GDM model are very close to 1 that again indicates very good liquid mixing in the reactor. The very good mixing of the batch in the RDC results from the discs rotation when the liquid adhere to the rough discs surface and from long mean residence time of the liquid in the reactor (3.4 – 28 hours). To test an influence of the speed of the discs rotation on reactor hydrodynamics the residence time distribution was also measured at 10 rpm and at the liquid volumetric flow rate 3 $cm^3\,min^{-1}$. Only negligible changes in values of the parameters of both models were observed. Contrary, the growth of the biomass at the discs surface exhibits extensive influence on the RDC hydrodynamics: after 36 days since the discs inoculation (1 week of a batch operation with subsequent continuous decolorization experiment) the residence time distribution was measured again with the discs heavily covered with the mycelium at the liquid flow rate 3 $cm^3\,min^{-1}$ and at the discs rotation speed of 3 rpm. Significant decrease of the mixing (a shift to plug-flow) was observed in experimental data (see Figure 2, full circles) and also via parameters of both models: for the TSBF model $n = 5$ and $b = 3.7$ was evaluated and for the GDM model $n = 1{,}47$ was estimated. A decrease of the mixing intensity can be ascribed to a smaller free liquid volume in the reactor due to the volume occupied by the mycelium.

Table 1: Parameters of flow models identified from tracer response data

Q [cm^3 min^{-1}]	TSBF*		GDM
	n	*b*	*n*
0.89	3	15.27	1.09
0.98	2	12.09	1.03
2.98	2	4.42	1.11
2.99	2	7.01	1.06
5.23	2	10.30	1.01
5.36	2	9.94	1.03
7.22	2	1.09	1.39
7.29	2	2.73	1.18

*) TSBF – Tanks in Series with Back-Flow Model
GDM – Gamma Distribution Model

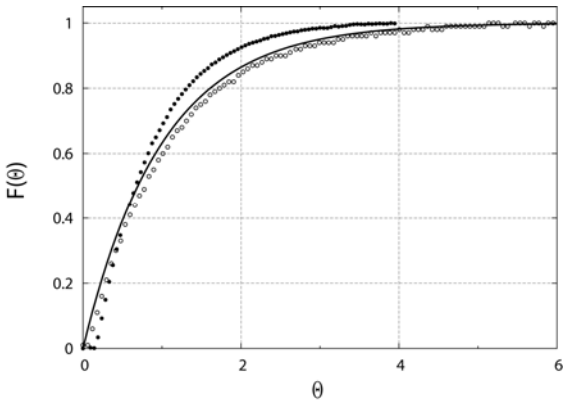

Figure 2: Comparison of experimental residence time distributions in RDC (○ – empty discs, Q=2.98 cm^3 min^{-1}; ● – mycelium covered discs, Q=3.07 cm^3 min^{-1}) with the RTD of the CSTR (line).

3.2 Decolorization experiments

After inoculation of the discs the RBC was operated in a batch mode at slow rotation speed using the Kirk's medium for 8 days to enable the mycelium to cover the surface of the discs. Then a series of continuous decolorization experiments was started using the RBBR dye dissolved in the Kirk's medium. The experiments were performed at 28 °C. The volumetric flow rate of the medium was set to 1 cm^3 min^{-1} and the RBBR inlet concentration to 100 mg dm^{-3}. At the 22nd day the volumetric flow rate and the dye concentration were changed to 0.2 cm^3 min^{-1} and 500 mg dm^{-3}, respectively. During the days 30 to 32 the flow rate and the inlet dye concentration were set to their initial values and then returned back to 0.2 cm^3 min^{-1} and 500 mg dm^{-3}. The experiments with the Kirk's medium continued to day 37, when the medium was switched to the malt extract one. The mass flow rate of the RBBR at the reactor inlet was kept at constant value (0.1 mg min^{-1}) during the entire experiment. The mass flow rate of the dye at the reactor outlet was always below 8×10^{-3} mg min^{-1}, i.e., the decolorization efficiency was always better than 92 % and the degradation rate of the RBBR was about 3.7 mg dm^{-3} h^{-1}. This value is slightly lower than the rate observed by Pocedič et al. (2010) in a batch mode due to higher mean dye concentration in the batch experiment. No noticeable differences in the degradation rate and in enzyme activities were noted with the malt extract medium compared to the Kirk's one, but the growth of the biomass was considerably faster.

After finishing the RBBR decolorization experiments the reactor operation was tested with the real textile waste water. However, only very low decolorization at the beginning of the experiment was observed, probably due to the lack of nutrients (the industrial waste water was only diluted with water and no nutrients were supplied) and due to its toxicity (high concentrations of dyes – cf. Section 2.2).

The activities of the enzymes participating in the dye decolorization were determined in the course of the decolorization experiments. Laccase activity varied from 1 to 10 U dm^{-3}, the MnP activity was usually below 1 U dm^{-3}. Pocedič et al. (2010) observed similar laccase activities, however, Tavčar et al. (2006) reported lower laccase activities (below 1.4 U dm^{-3}) and higher MnP activity under very similar operating conditions with the Reactive Orange 16 dye. No LiP activity was detected in our experiments similarly to the experiments of Kasinath et al. (2003).

4. Conclusions

The residence time distributions were determined experimentally to characterize hydrodynamics of the RDC reactor under various operating conditions. The experimental results proved very high intensity of the liquid phase backmixing in the RDC. An extent of the backmixing was quantified by parameters of two flow models – the tanks in series model with backflow and the gamma-distribution model – by fitting the models to measured tracer data. The extent of the liquid phase backmixing is predominantly influenced by the liquid flow rate and only to quite a low level by the rotational speed of the discs. The

covering of the discs surface with the mycelium significantly decreases the degree of the backmixing. The decolorization experiments with the RDC using the immobilized *Irpex lacteus* mycelium and operated in the continuous flow-through mode proved that this reactor is capable to decolorize anthraquinone textile dyes for long time periods with a high efficiency (≥92%). The growth medium composition affects the growth rate of the fungus but not the decolorization. An abundant mycelium growth in the reactor was observed that resulted to serious problems during the long term operation. Optimization of the amounts of nutrients in the water will be therefore investigated in a future.

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Notation:

b	parameter of the TSBF model	n	parameter of the TSBF model
c_{in}	tracer inlet concentration	n	parameter of the GDM model
c_k	tracer concentration in the n-th cell	Q	volumetric flow rate
$f(\theta)$	res. time probability density function	Γ	gamma function
$F(\theta)$	residence time distribution function	θ	dimensionless residence time

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