Rotating drum biological contactor and its application for textile dyes decolorization

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Abstract

A laboratory-scale rotating drum biological contactor (RDBC) with immobilized white rot fungus *Irpex lacteus* was used to decolorize synthetic wastewaters containing the azo dye Reactive Orange 16 (RO16) and the anthraquinone dye Remazol Brilliant Blue R (RBBR). Two particulate drum packings were used to immobilize the fungus: the AL-Schwimmbett Medium 100L that appeared not to be convenient, because of a low adhesion of the fungal mycelium to the particles, and the oak wood cylinders that supported formation of a thin layer of the fungal biofilm on the surface of the particles resulting to faster degradation of the dyes in comparison with the AL-Schwimmbett Medium 100L particles. In batch decolorization experiments at the initial dyes concentration of 100 mg dm\(^{-3}\) it took from 4 to 5 hours for the RBBR and 21 hours for RO16 to get 85% decolorization. In the continuous decolorization experiment at high inlet mass flow rate (0.5 mg min\(^{-1}\)) of the RBBR dye the degree of decolorization achieved was just 20%. Activities of the enzymes manganese-dependent peroxidase, lignin peroxidase and laccase were also monitored during experiments.

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1. Introduction

Textile industries produce huge amounts of wastewaters. These wastewaters contain synthetic textile dyes of various kinds, which are frequently carcinogenic, mutagenic, teratogenic, and toxic or can affect the aquatic environment in many other negative ways [1]. Wastewater treatment procedures used in common municipal wastewater treatment plants are not capable to remove efficiently the dyes from the wastewaters and these harmful substances are then released to water courses. Therefore, it is necessary to modify contemporary wastewater treatment plants to be more efficient in dyes removal. Besides some physical or chemical methods (adsorption, ozonation, oxidation, flocculation etc.), it is also possible to use biological methods for dyes removal from the waters. The biological methods are usually considered to be less expensive and quite effective in degradation of persistent pollutants including the dyes [2]. In last years, white rot fungi have been frequently studied for these purposes because of their ability to decompose a variety of organic compounds including the synthetic dyes. The white rot fungi produce ligninolytic enzymes such as laccase, manganese-dependent peroxidase and lignin peroxidase, which are able to degrade wide range of chemical compounds. In addition to the ligninolytic enzymes, other enzyme systems produced by the fungi can also participate to dyes degradation. For example, Rodríguez et al. [3] observed that Pleurotus ostreatus culture was able to decolorize 12 of 23 industrial textile dyes, whilst crude extracellular extracts were able to decolorize only 5 dyes. A number of white rot fungi species (e.g., Phanerochaete chrysosporium, Trametes versicolor, Pleurotus ostreatus) can be used to decolorize textile wastewaters. In this study the white rot fungus Irpex lacteus was used. This fungus proved to decolorize textile dyes under various process conditions [4]. Moreover, this fungus is highly resistant to microbial contamination. According to Novotný et al. [5] Irpex lacteus was able to colonize both sterile and non-sterile soil and they also observed good colonization of a non-sterile soil contaminated with PAH’s and heavy metals.

When microorganisms are used for decolorizations, it is usually favorable to use them in an immobilized form. Immobilized cells are usually more resistant to both biotic and abiotic stresses, toxic substances and sometimes exhibit more intense survival and metabolic activities [6]. Polyether and polyurethane reticulated foams or natural organic materials like wood or straw are commonly used to immobilize Irpex lacteus. In case of the fungus Irpex lacteus fluidized bed reactors, trickle bed reactors or rotating biological contactors (RBC) were often used for wastewater decolorizations. A standard RBC consists of circular discs mounted on a shaft, which are partially or totally immersed in a treated liquid. The rotation of discs ensures mixing and aeration of the liquid and supplementation of oxygen and nutrients to microorganisms immobilized on the surface of the discs. RBC also provides several methods (e.g., change of rotation speed or direction) to control biofilm thickness and is more resistant to clogging by solids. These factors make the RBC more suitable for an industrial utilization than other types of reactors as confirmed, for example, by Novotný et al. [7]. According to their results the RBC is less efficient in dye decolorization, but more resistant to clogging compared to the trickle bed reactor.

Despite our own research results (not published yet) confirmed that RBC with the fungus Irpex lacteus immobilized on discs is capable of efficient long time decolorization of textile dyes, the problems related to the excessive growth of the fungal mycelium were encountered. The abundant mycelium growth may change reactor operational characteristics and after certain time period it could result in full clogging of the device. Therefore, we tried to use another type of RBC: a Rotating Drum Biological Contactor (RDBC). In this reactor type a drum mounted on a central shaft is used instead of the discs. The drum is usually made of a wire mesh and is partially or completely filled with randomly ordered packing particles, which are used for mycelium immobilization. By variations of the speed of the drum rotation and of the amount of particles in the drum, shear stresses and therefore also thickness of the fungal biofilm on packing particles in the drum can be controlled. A choice of kind of packing particles used for fungus
immobilization is of high importance. Domínguez et al. [8] demonstrated that the RDBC is suitable for white rot fungi cultivation. They precultivated the fungus *Phanerochaete chrysosporium* on cubes of a fibrous nylon sponge and then transferred the cubes to the drum of a RDBC. The RDBC operated and produced high levels of ligninolytic enzymes for at least 17 days.

The aim of this study was to construct a laboratory scale RDBC suitable for textile dyes degradation by white rot fungus *Irpex lacteus*. A development of a procedure of reactor inoculation with the fungus securing that the most of the mycelium is attached to the packing particles in the drum and not suspended in the liquid was performed. Several kinds of packings were tested to find the most convenient one. The effectivity of the RDBC was tested by decolorizations of synthetic wastewaters containing textile dyes.

2. Materials and methods

2.1. Microorganism

White rot fungus *Irpex lacteus* (strain Fr. 238 617/93, isolated from the forests of Czech Republic) was used in experiments. The strain was obtained from the Culture Collection of Basidiomycetes (CCBAS) of the Academy of Sciences of the Czech Republic.

2.2. Chemicals

The anthraquinone dye Remazol Brilliant Blue R (RBBR) and the azo dye Reactive Orange 16 (RO16) were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and were obtained from local sources.

2.3. Culture media

The solid agar medium with 0.5% (w/v) malt extract, 1% (w/v) glucose and 2% (w/v) nutrient agar [7] was used to maintain and store the fungus. Kirk medium with low nitrogen content (0.1 g dm⁻³) [9] was used for fungus cultivation and in decolorization experiments. The media were sterilized at 121°C for 20 minutes; pH value was kept at 4.5.

Fig. 1. Schematics of the Rotating Drum Biological Contactor used in experiments
2.4. Rotating drum biological contactor

The rotating drum biological contactor used in this work consisted of the drum (13 cm diameter, 25.5 cm length) made of a stainless steel mesh (3 mm mesh size) was divided into 3 sections by polycarbonate dividing walls (cf. Fig. 1). Volume of each section of the drum was 1021 cm$^3$. The drum was mounted on a stainless steel shaft and placed in a round-bottomed vat. The inner dimensions of the reservoir were: 31.2 cm length, 16.2 cm width and 15.6 cm height. The total inner volume of the reactor was $7 \times 10^{-3}$ m$^3$ and the filling liquid volume was $1.5 \times 10^{-3}$ m$^3$. The drum was filled with randomly arranged particulate packings: the AL-Schwimmbett Medium 100L (Aqualogistik, Germany) or the oak wood cylinders (diameter 8 mm, length 40 mm). The reactor was equipped with liquid and gas inlet and outlet ports (see Fig. 1). The drum was rotated by a PC controlled stepper motor.

2.5. Reactor inoculation

First, the drum packing particles were thoroughly washed with hot distilled water to remove soluble residues and RDBC was steam sterilized at 121°C for 20 minutes. The packing particles were inoculated with the homogenized *Irpex lacteus* mycelium prepared in the following way: Three circular targets (diameter 1 cm) of the fungus grown on the solid agar medium were aseptically transferred to an Erlenmeyer flask with 50 cm$^3$ of the Kirk medium and then the fungus was cultivated at 28 °C in a static culture. After 7 days of cultivation the entire content of the flask was homogenized by the ULTRA TURRAX T18 (IKA, Germany) homogenizer [10] and the homogenate were used for particles inoculation.

Two ways were used to inoculate the reactor packed with the AL-Schwimmbett Medium 100L particles. The first way consisted in spreading the homogenate (15 cm$^3$ to each section of the drum) over the packing particles already placed in the drum (75 g of the particles within each section of the drum, approximately a half of the drum volume was filled with the particles). Then the reactor was filled with 1.5 dm$^3$ of the Kirk medium, sealed and placed to a closed box thermostated at 28 °C to allow the growth of the fungus on the surface of the particles. The drum was rotated at 3 rpm. The second way of inoculation (used also to inoculate the oak wood cylinders) consisted in these steps: The fungus was precultivated on packing particles in static Erlenmeyer flasks containing 30 cm$^3$ of the Kirk medium and 37.5 g of packing particles (50 cm$^3$ and 40 g, respectively in case of the oak wood cylinders). The particles were sprayed with 10 cm$^3$ of homogenized mycelium prior precultivation in the flasks. After 7 days of static cultivation at 28°C (11 days in case of the oak wood cylinders) the particles without liquid medium were aseptically transferred to the drum (contents of 2 flasks were placed into each section of the drum). Then the RDBC was operated in a batch mode for at least one week at 28 °C to allow biofilm development. The reactor was filled with 1.5 dm$^3$ of Kirk medium and the drum rotated at 3 rpm.

2.6. Dye concentration and enzyme activity assays

The concentration of dyes was determined by measuring the absorbance of liquid samples at 592 nm for RBBR and at 490 nm for RO 16.

The lignin peroxidase (LiP) activity was measured by veratryl alcohol assay [11]. One unit (U) of LiP activity is defined as the amount of the enzyme consuming 1 μmol of substrate per minute. The activity of manganese-dependent peroxidase (MnP) was determined by DMAB/MBTH (3-dimethylaminobenzoic acid/3-methyl-2-benzothiazoline hydrazone hydrochloride) assay [11] and the activity of laccase by ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) assay [12]. One unit of activity of these enzymes is defined as the amount of enzyme producing 1 μmol of the product per minute. The enzyme activities were measured at 28°C.
3. Results and discussion

In the first experiment the RDBC with the particulate packing (AL-Schwimmbett Medium 100L) inoculated directly in the drum was used. After one week of incubation the particle surface was almost completely free of the mycelium (see Fig. 2a), but the mycelium attached to other parts of the RDBC, especially to the stainless steel mesh of the drum and to the shaft, was clearly apparent. Therefore, this way of the reactor inoculation was evaluated as unacceptable and the experiment was terminated. No decolorization experiments were attempted. The most probable cause of the ineffective inoculation of the packing in the reactor is low adhesion of the fungal mycelium to the surface of the AL-Schwimmbett Medium particles and their nonporous structure not supporting mycelium attachment during inoculation.

To secure the particles coverage with the fungal mycelium the fungus in the subsequent experiment was precultivated in static cultures in Erlenmeyer flasks with the packing particles (cf. Section 2.5). It resulted in sufficient coverage of the particles surface with the mycelium (cf. Fig. 2b) and then the particles were transferred to the RDBC. However, after 7 days of cultivation in the RDBC the coverage of the particles with the mycelium was not satisfactory (Fig. 2c). The reason of this observation is, probably, the low adhesion of the mycelium to the particles as in the first experiment. Despite this observation two batch RBBR decolorization experiments were carried out at the initial dye concentration of 100 mg dm\(^{-3}\). In the first decolorization experiment the dye decolorization was very slow: it took 3 days to reach 85 % decolorization. Seven days since the start of the first decolorization experiment the next has been started using the same mycelium attached to the particles. The decolorization was significantly faster (90 % decolorization within 23 hours). This acceleration was probably caused by considerable growth of the fungus on a surface of all inner parts of the reactor except of the packings itself (Fig. 2d). These results can be compared to the observations of Pocedič et al. [10]. Under similar conditions, they reached in a
rotating discs biological contactor with the discs made of a polyether foam 90 % decolorization in about 15 hours and with discs made of the pine wood even in less then 5 hours. Because of low adhesion of the fungal mycelium to the AL-Schwimmbett Medium particles and therefore low achievable decolorization rates this mycelium carrier did not proved to be convenient and perspective drum packing.

The oak wood cylinders appeared to be more suitable packings for the RDBC. During the entire experiment with this kind of packing, there was a thin biofilm of the mycelium observable on the surface of the cylinders. However, an excessive growth of the fungus on the mesh of the drum and in the liquid within the reactor was still observable. Several batch decolorization experiments with the RBBR and RO16 dyes at the initial dye concentrations of 100 mg dm$^{-3}$ were performed. It took from 4 to 4.5 hours to reach 85 % decolorization of the RBBR. To quantify contribution of the mycelium suspended in the liquid to the total decolorization rate, the liquid medium with suspended mycelium was poured out of the reactor and fresh (mycelium free) Kirk medium with the RBBR was dosed to the reactor. Only slight decrease of the decolorization rate was observed: it took 5 hours to get 85 % decolorization. These values are comparable to those observed by Pocedič et al. [10]. In experiment with the RO16 dye it took at least 21 hours to get 85 % decolorization. Pocedič et al. [10] also observed slower decolorization rate of RO16 in comparison with RBBR. However, the decolorization rate observed in our reactor was very low. For example, Tavčar et al. [7] reached much faster decolorization rate of RO16 in rotating discs reactor with immobilized Irpex lacteus: 85 % decolorization was reached in about 6 hours even at higher initial dye concentration (150 mg dm$^{-3}$).

A single continuous decolorization experiment with the RBBR dye and with the drum packed with the oak wood cylinders was carried out, too. The inlet RBBR concentration and volumetric flow rate were 100 mg dm$^{-3}$ and 5 cm$^3$ min$^{-1}$, respectively. This experiment was not performed under strictly sterile conditions and Kirk medium without DMS and inlet pH value 5.5 was used. After 40 hours of continuous operation the outlet RBBR concentration stabilized at 80 mg dm$^{-3}$. It corresponds to the degradation rate of 4 mg dm$^{-3}$ h$^{-1}$. Slightly lower decolorization rates (2.3 – 3.6 mg dm$^{-3}$ h$^{-1}$) were observed by Pocedič et al. [13] in a trickle-bed reactor with Irpex lacteus immobilized on various carriers and almost the same degradation rate was observed in rotating disc reactor in our previous experiments but at lower inlet dye concentration [14].

![Fig. 3. Time traces of enzyme activities in the RDBC reactor in source of RBBR decolorization](image-url)
The enzyme activities of MnP and laccase were significantly higher in the RDBC packed with the oak wood cylinders than in the reactor packed with the AL-Schwimmbett Medium (Fig. 3). The reason of this observation is the presence of the wood and lignin in the reactor invoking higher secretion of the enzymes. Pocedič et al. [10] observed higher laccase activity in the rotating biological contactor with wooden discs, too. Contrary, Kasinath et al. [15] observed higher enzymes activities in a packed-bed reactor with the fungus *Irpex lacteus* immobilized on polyurethane foam compared to the pine wood cubes and higher enzyme activities could result from a presence of rather more biomass in the reactor with the wooden mycelium carriers. No LiP activity was noted in any experiment. In the case of RO16 dye Novotný et al. [16] and Kasinath et al. [15] did not observe any LiP activity in stationary cultures or in packed-bed bioreactor with fungus *Irpex lacteus* but in the presence of the RBBR dye high levels of the lignin peroxidase were detected and MnP activity was significantly higher in both studies. Pocedič et al. [10] observed comparable laccase activities in the rotating discs biological reactor.

4. Conclusions

In this study experimental laboratory-scale rotating drum biological contactor was constructed. Two particulate drum packings and two ways of reactor inoculation with the fungus were used. Spraying of packing particles with the mycelium homogenate directly in the reactor was not observed as an applicable way of inoculation due to poor covering of packing particles with the fungal mycelium and due to fast growth of fungus on the inner parts of the reactor. Precultivation of fungus on packing particles in static cultures in Erlenmeyer flasks with subsequent transfer to the RDBC proved as successful inoculation method. Al-Schwimmbett Medium 100L did not prove as suitable drum packing, due to low adhesion of the mycelium to particles. Only very low decolorization rates in the batch decolorization experiments were observed with the RBBR dye. The packing particles made of the oak wood cylinders yielded notably better results. Thin biofilm was observed on the surface of the particles. Decolorization rates of the RBBR dye reached in the RDBC filled with the oak wood cylinders were close to the rates observed in the rotating discs biological reactor [10]. In the case of the RO16 dye the decolorization rates gained in this study were lower compared to the rotating discs reactor [7].

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