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Rotating Drum Biological Contactor for Immobilization of the White-rot Fungus *Irpex Lacteus* and Degradation of Textile Dyes

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The white-rot fungus *Irpex lacteus* is able to decolorize a wide range of synthetic textile dyes. The aim of this study was a verification of this capability when the decolorization takes place in a medium without addition of an easily degradable carbon source, such as glucose. The decolorization ability was verified by degradation of the azo dye Reactive Orange 16 (RO16) in a laboratory scale Rotating Drum Biological Contactor (RDBC). The drum was filled by a mixture of straw (source of nutrients) and Al-Schwimmbett® plastic particles. Two sets of batch decolorization experiments with synthetic wastewater consisting of tap water and dye RO16 (initial concentration 100 mg.dm^{-3}) were performed. A decrease of the degradation rate of the dye RO16 in subsequent sets of batch degradation experiments was observed. In the continuous experiments with the tap water based medium (input RO16 concentration 50 mg dm^{-3}) the decolorization efficiency was very low – less than 20 % within 15 days. When glucose was added to the input stream (at concentration of 5 g dm^{-3}), high degradation efficiency was observed – more than 95 % on 14th day. Besides the higher dye degradation rate, the other impact of the glucose addition to the medium was the overgrowing of the fungal biofilm. It resulted in a significant decrease of liquid volume in the reactor and certain operational problems, such as clogging of the liquid output with the biomass.

1. Introduction

Textile industry is a significant source of waste waters, which are usually highly coloured. Typical dye concentration in outlet streams from textile industries varies within a range from 10 to 200 mg.dm^{-3}. The chemical composition of these streams may change very quickly and substantially as a consequence of actual steps adopted in a dying process (Doble and Kumar, 2005). As the most of synthetic dyes are harmful for environment, it is necessary to remove or degrade them before the wastewaters are released to watercourses. However, applications of traditional wastewater treatment technologies to remove synthetic dyes are highly ineffective, due to chemical stability of the dyes (Forgacs et al., 2004). Therefore there are numerous attempts to improve current treatment technologies or develop new ones, which would be more efficient and possibly also cheaper.
White rot fungi are known to degrade a wide range of synthetic dyes as shown by Novotny et al. (2001) on a range of chemically different dyes and later by Nilsson et al. (2006), who performed batch and continuous tests with Reactive Blue 4 and Reactive Red 2. The most frequently used white-rot fungi species are *Pleurotus ostreatus*, *Trametes versicolor*, *Phanerochaete chrysosporium* or *Irpex lacteus*. In our study the fungus *Irpex lacteus* was used, as it showed remarkably high potential to decolorize a broad spectrum of synthetic dyes under diverse growth conditions (Novotny et al., 2004b). The high capability of white rot fungus to degrade the dyes is often associated with the production of non-specific extracellular ligninolytic enzymes (e.g., laccase, manganese-dependent peroxidase or lignin peroxidase). However, the

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production of these lygninolytic enzymes by *Irpex lacteus* is very low in a comparison with other white rot fungi and it still keeps high degradation rate of dyes (Novotny et al., 2004).
The most of dye degradation experiments were carried out using solid agar media and in shaken or static flask cultures. However, for industrial applications these methods are markedly insufficient. The only few attempts to use white rot fungi in larger scale reactors – for example: a trickle bed reactor (Pocedič et al., 2009), a rotating disc biological contactor (Tavčar et al., 2006) or a rotating drum biological contactor (Domínguez et al., 2001) were performed. The most of the degradation experiments were performed using growth media or, at least, an addition of a source of easily degradable organic carbon (e.g., glucose or fructose) to the treated liquid, which makes the remediation procedure remarkably expensive. Therefore, we tried to immobilize the white-rot fungus *Irpex lacteus* on a straw that may serve as a quite cheap source of nutrients and a solid mycelium carrier simultaneously and use it for a dye decolorization in a laboratory scale rotating drum biological contactor.

2. Material and methods

2.1 Microorganism

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

2.2 Chemicals

The azo dye Reactive Orange 16 (RO16) and 2,2'-dimethylsuccinic acid were purchased from Sigma-Aldrich. All other chemicals used in the experiments were of the analytical grade and were obtained from local sources.

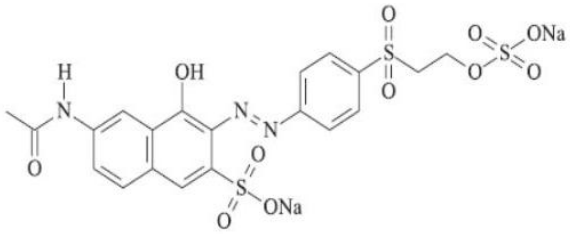

Figure 1: Molecular structure of Reactive orange 16 (Gomes et al., 2011)

2.3 Growth media

Solid agar media consisting of nutrient agar (20 g.dm^{-3}), malt extract (5 g.dm^{-3}), glucose (10 g.dm^{-3}) and distilled water was used for storage of fungal mycelium. Low nitrogen Kirk's medium containing ammonium tartrate (0.1 g.dm^{-3}) as a source of nitrogen and 2,2'-dimethylsuccinic acid (1.46 g.dm^{-3}) as a buffer was used for precultivation of the fungus in Erlenmeyer flasks (Pocedič et al., 2009). The pH value of the Kirk's medium was set to 4.5. All media were sterilized in an autoclave at 121 °C for 20 minutes.

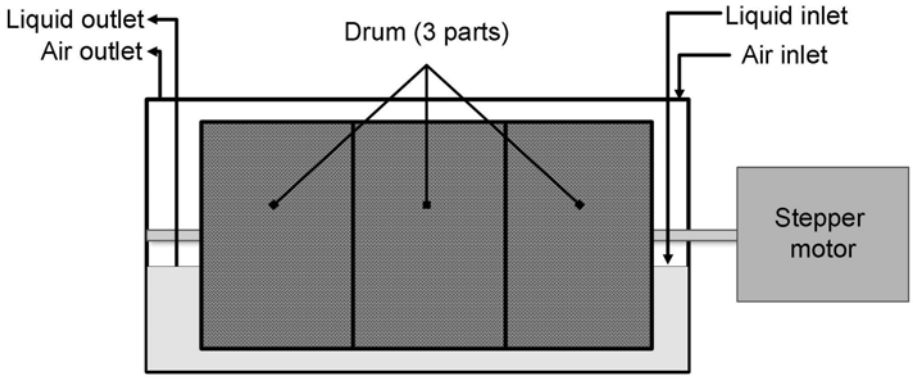

Figure 2: Schematics of the rotating drum biological contactor used in experiments

2.4 Rotating drum biological contactor

A home-made laboratory scale Rotating Drum Biological Contactor (RDBC) was used in this work (see Figure 2). The frame of the drum (25.5 cm length, 13 cm diameter) was made of polycarbonate plates. This frame was covered by a stainless steel mesh (mesh size 3 mm). The drum was divided to 3 sections by polycarbonate walls and was filled by a mixture of randomly arranged Al-Schwimmbett® plastic particles (half of the drum volume) and the straw. Volume of each section was 1021 cm^3. The drum was placed in a round-bottomed vat (31.2 cm length, 16.2 cm width, 15.6 cm height). The inner volume of the vat was 7 dm^3, the liquid volume in the reactor was 1 dm^3. The reactor was sealed and equipped by liquid and gas inlets and outlets. The drum was mounted on a stainless steel shaft and was rotated by a PC controlled stepper motor.

2.5 Reactor innoculation

Prior the inoculation the Al-Schwimmbett® plastic particles were thoroughly washed with hot distilled water to remove soluble residues and the whole reactor was sterilized in an autoclave at 121 °C for 20 minutes. Each section of drum was inoculated with 15 cm^3 of homogenized *Irpex lacteus* mycelium. The mycelium homogenate was prepared in following way: Erlenmeyer flask with 50 cm^3 Kirk's medium was inoculated by three circular targets of mycelia grown on the solid agar medium. After one week of the static cultivation at 28 °C, the content of the flask was homogenized by ULTRA TURRAX T18 homogenizer.

2.6 Analytical assays

The concentration of RO16 in liquid samples was measured spectrophotometrically at 490 nm. Laccase activity was determined by 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay (Kunamneni et al, 2008). One unit (1 U) of laccase activity is defined as an amount of enzyme necessary to produce 1 µmol of ABTS cation radical per 1 minute (ε_{420} = 36.000, Childs and Bardley, 1975). Glucose concentration was determined by glucose kit and standard from BioSystems.

3. Results and discussion

3.1 Batch decolorization experiments

After inoculation with the fungus the RDBC reactor was operated in a batch mode for 14 days to allow for an extensive growth of the mycelium both on the straw and the plastic particles. The reactor was filled with 1 dm^3 of Kirk's medium, operated at the temperature of 28 °C and the drum was rotated at 3 rpm. At the start of each batch decolorization experiment the liquid in the reactor was replaced with 1 dm^3 of the synthetic waste water consisting of tap water, 2,2'-dimethylsuccinic acid (1.46 $.dm^{-3}$), dye RO16 (100 $mg.dm^{-3}$), pH of the liquid was set to 4.5. At first, the set of 3 batch decolorization experiments was carried out. The results of these experiments are shown in Figure 3. Decreasing degradation rate of the dye is apparent. In the first batch we got 78 % decolorization within 5 hours, but in the third batch only 65 % decolorization was observed within the same period. Pocedič et al. (2010) degraded the RO16 dye (initial concentration 100 $mg.dm^{-3}$) in a rotating disc biological contactor containing 12 polyether foam discs. This reactor was geometrically similar to RDBC used in this study and it took about 50 hours to get 80 % decolorization. The higher degradation rate in our case may be caused by a larger surface in of the particles and the straw a drum, which is available for fungus attachment or (more probably) by the presence of the straw as a natural source of nutrients. Pocedič et al. (2010) observed higher degradation rate of the dye Remazol Briliant Blue R, when they used discs made of a pine wood, despite these discs have smaller surface than porous polyether foam. Novotny et al. (2004) reported higher degradation rate of the dye RBBR in a packed bed reactor by *Irpex lacteus*, when they used a pine wood instead of the polyurethane foam, too. The degradation of dye RO16 by white rot fungus in RDBC can be described by the first order kinetics. From a comparison of the reaction rate constants (Figure 4) the decrease of the degradation rate is also obvious especially between the first and the third batch.

After the first set of batch experiments the fungus in RDBC was regenerated by a new batch of Kirk's medium. This regeneration took 4 days and then a new set of batch decolorization experiments was carried out. The degradation rate constant for the first batch was even higher than degradation rate constant for the first batch before regeneration (see Figure 4). This increase of degradation rate can be caused by a growth of the biofilm in the reactor during the regeneration. The degradation rate during the second batch after the regeneration was even higher. There were probably nutrients retained in the straw or in the biofilm. However, after the nutrients exhaustion (during the third and the fourth batch experiments) the degradation rate decreased again. During the fourth batch it took even 26 hours to get only 78 % decolorization.

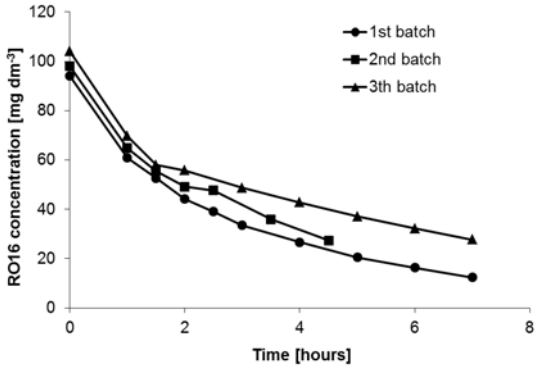

Figure 3: 1st set of batch decolorization experiments

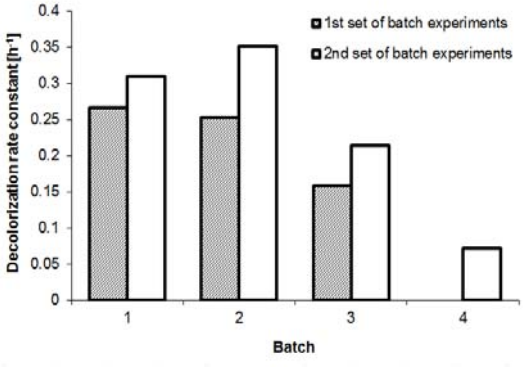

Figure 4: Decolorization rate constant for the dye RO16 in batch experiments

3.2 Continuous decolorization experiments

After the end of batch decolorization experiments, several continuous decolorization experiments in DRBC were performed. The inlet volumetric liquid flow rate was 0.5 $cm^3.min^{-1}$. Before start of each experiment, the fungus mycelium in the reactor was regenerated by a new batch of the Kirk's medium. During the first two experiments a synthetic waste water consisting of tap water and the dye RO16 (50 mg dm^{-3}) was used.

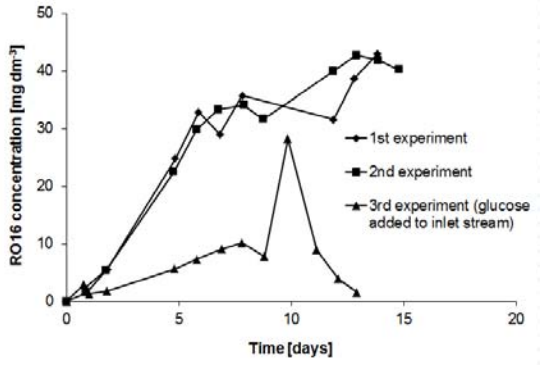

Figure 5: Continuous decolorization experiment

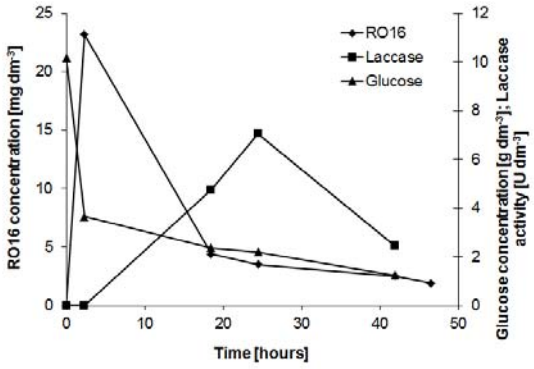

Figure 6: Regeneration before the beginning of 3rd continuous experiment

In Figure 5 the time dependence of the dye concentration in the RDBC is depicted. The dye concentration in the reactor was slowly increasing. On 14th day since the beginning of the experiments the dye concentration in the outlet of the RDBC was higher than 40 $mg.dm^{-3}$ (decolorization efficiency less than 20 %). These results correspond to the batch decolorization experiments. Decrease of the degradation rate was caused both by the consumption of the nutrients and by washing-out of the nutrients from the reactor. When the glucose was added (5 $g.dm^{-3}$) to the inlet stream of the synthetic wastewater into the reactor, higher degradation rate was observed during the entire experiment (see Figure 5). On the 10th day a higher dye concentration in the liquid outlet was noted. It was caused by a clogging of the liquid outlet by mycelium and by inundating of the reactor by the treated liquid (during the continuous decolorization experiments stable liquid level in RDBC was maintained). When the problem was fixed and the liquid level stabilized, the outlet dye concentration decreased again and on 14th day the decolorization efficiency was even higher than 95 % (it corresponds to the degradation rate of RO16 of 1.425 $mg.h^{-1}$). The average glucose consumption for this period was 0.1 $g.h^{-1}$. The experiment continued even after 14th day, but the problems with the liquid output clogging were frequent and the output dye concentration was steeply fluctuating. Therefore the experiment was stopped. From these results we can deduce that the glucose is necessary to achieve sufficient decolorization of the dye RO16 by *Irpex lacteus* in our system.

Very interesting is to observe laccase activity, glucose and dye concentration after the mycelium regeneration between continuous decolorization experiments (Figure 6). After replacing the liquid in the

reactor by Kirk's medium, the dye RO16 was quickly desorbed from the mycelium back to the liquid and degraded. It confirms presumption that substantial amount of nutrients remain in the reactor after liquid replacement. We also observed very fast consumption of glucose and the activity of laccase was indicated. The maximum activity of laccase was noted usually one day since media replacing. Tavčar et al. (2006) reported lower laccase activity (below 1.4 U dm^{-3}). Novotný et al. (2004) noted in trickle-bed containing *Irpex lacteus* immobilized on pine wood or polyether foam similar laccase activities during batch experiments. During the decolorization experiments in RDBC there was usually null laccase activity within eigth days since beginning of experiment despite glucose was added to the liquid inlet. The Kirk's medium obviously induces higher production of the extracellular laccase by *Irpex lacteus*.

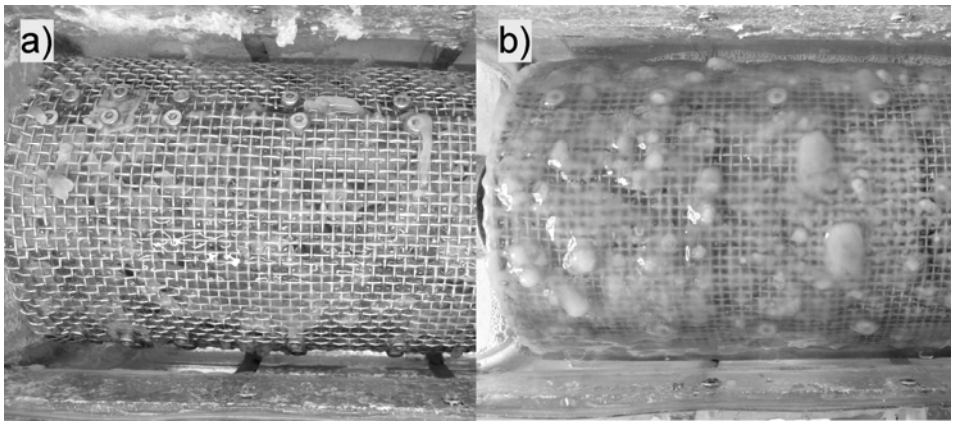

Figure 7: Snapshots of the drum in RDBC within 3rd continuous decolorization experiment (glucose added to inlet liquid stream: a) before start of the experiment, b) at the end of the experiment (after 25 days)

Presence of glucose in the reactor induces growth of the biofilm. During the batch and continuous decolorization experiments without added glucose there was negligible change of liquid volume in the RDBC. However, during mycelium regeneration we observed reduction of the liquid volume (1st regeneration: 950 → 900ml, 2nd regeneration: 900 → 800 ml, 3rd regeneration: 800 → 750 ml). In the continuous experiment with added glucose the biofilm growth and liquid volume reduction was very significant (750 → 500 ml), too. Volume reduction is also obvious when we compare snapshots of the inside of the RDBC before and after the experiment (Figure 7). At the end of fungus cultivation in the RDBC the drum was fully covered with the biofilm. There was also a biofilm attached to some parts of the bottom and the walls of the reactor. Inside the drum the straw was covered with biofilm, but there was only a thin biofilm attached to the plastic particles and they were still free for liquid passage (Figure 8). It corresponds to our previous report, that adhesion of fungus *Irpex lacteus* to these particles is very poor (Sima et al., 2012). The free way for liquid flow in a drum can support liquid exchange and therefore increase the degradation rate of the dye. However, this fact has to be verified by another research.

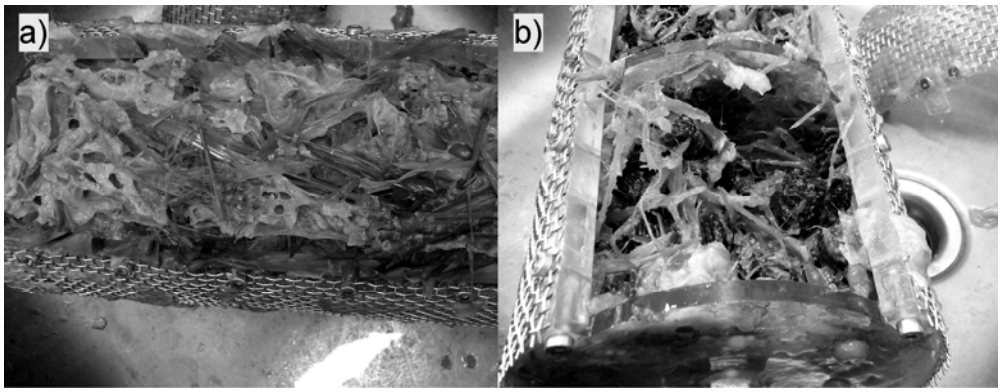

Figure 8: Snapshots of the inside of the drum of RDBC at the end of fungal cultivation

4. Conclusions

The results reported in this paper demonstrate applicability of the rotating drum biological contactor (RDBC) containing the fungus *Irpex lacteus* immobilized on the support consisting of a mixture of the biological material (straw) and the Al-Schwimmbett® plastic particles to dye degradation. However, the straw – the natural source of nutrients, is not sufficient to keep high decolorization rate of the dye RO16. The adding of glucose to the treated liquid seems to be necessary to keep the long time operation. The consumption rate of glucose is very low. In continuous decolorization experiments the most serious problem represents abundant growth of the fungus biofilm within the RDBC, which caused clogging of the liquid outlet. It is the typical problem of operation of bioreactors containing white-rot fungi in a nutrient rich environment. This problem has to be solved before possible industrial application of the RDBC and white-rot fungi. The presence of Al-Schwimmbett® plastic particles in the drum of the reactor appeared to be beneficial for its long time operation.

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