ARCHITECTURE CIVIL ENGINEERING ENVIRONMENT
The Silesian University of Technology No. 1/2017

THE POSSIBILITY OF USING OF *HYPHOLOMA FASCICULARE* MYCELIUM IN DECOLORIZATION OF ANTHRAQUINONE DYE RBBR

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Received: 19.07.2016; Revised: 4.09.2016; Accepted: 20.09.2016

Abstract
The aim of this study was to determine the usefulness of two fungal strains of *Hypholoma fasciculare* (L1 and L3) for effective decolorization of anthraquinone dye RBBR (remazol Brilliant Blue R). The main part of the work was concentrated on assessment of the influence of immobilization of biomass on the efficiency of RBBR removal. Zoo- and phytotoxicity of after process solutions were evaluated. Differences in the dye removal effectiveness between strains were observed. Decoloration of dye was more efficient in samples with mycelium immobilized on a polypropylene foam, what probably was associated with increased enzyme activity of the strains, as well as enhancement of the contact of the dye with the mycelium. Strain L3 respectively removed 100% (mycelium immobilized) of the dye after 24h and 95.8% (mycelium suspended) of the dye after 96h. For complete removal of the dye the immobilized biomass of strain L3 needs 24 hours of incubation, and L1 48h. Strain L1 completely removed the color after 96 h of the experiment, regardless of whether the biomass has been immobilized or not. RBBR dye was not toxic to *Daphnia magna*. The zootoxicity test indicated that usage of both strains of *Hypholoma fasciculare* in the discoloration of the dye RBBR is safe for the environment, since even at the highest concentrations of after processes solutions were not observed immobilization effect of *Daphnia magna*. In the case of phytotoxicity it has been reduced from class III to I.

Streszczenie
Celem badań było określenie możliwości wykorzystania do dekoloryzacji antrachinonowego barwnika RBBR (remazolowy błękit brylantowy R) dwóch szczepów grzybów *Hypholoma fasciculare* (L1 i L3). Główna część badań skupiała się na ocenie wpływu immobilizacji biomasy na efektywność dekoloryzacji RBBR. Po zakończeniu badań przeprowadzono testy zoo- i fitotoksyczności. Obserwowano różnice w efektywności dekoloryzacji między oboma szczepami. Dekoloryzacja barwnika zachodziła lepiej w próbach z grzybnią immobilizowaną na polipropylenowej gąbce, co wiąże się prawdopodobnie ze zwiększeniem aktywności enzymatycznej szczepów, a także z polepszeniem kontaktu barwnika z grzybnią. Szczep L3 usunął odpowiednio 100% (grzybnia immobilizowana) i 95.8% (grzybnia zawieszona) barwnika po 96h. Na całkowite usunięcie barwnika w przypadku immobilizowanej biomasy szczepu L3 wystarczyło 24h inkubacji, a dla szczepu L1 48h. Szczep L1 całkowicie usunął RBBR po 96h eksperymentu, niezależnie od tego czy grzybnia była immobilizowana czy nie. Barwnik RBBR nie jest toksyczny dla *Daphnia magna*. Badania zootoksyczności wskazały, iż oba szczepy mogą zostać wykorzystane w bezpieczny sposób do dekoloryzacji tego barwnika, ponieważ nawet w najwyższym stężeniu nie obserwowano unieruchomienia *Daphnia magna*. W przypadku fitotoksyczności uległa ona obniżeniu z klasy III do I.

Keywords: **Decolorization; Ecotoxicity; *Hypholoma fasciculare*; RBBR; Phytotoxicity; Zootoxicity.**

1. INTRODUCTION

The industrial production involves generation of a number of hardly biodegradable substances with the aromatic structure (such as PAH, pesticides, dyes). Only the appropriate enzyme system with low specificity and high redox potential creates the possibility of removing them from the wastewater and environment [3, 6, 8]. Synthetic dyes are compounds with the aromatic structure, and may cause a significant threat to the aquatic environment. The dyes most commonly used in the industry are azo, triphenylmethane or anthraquinone [11-12]. All dyeing processes are associated with pass of the residues of unused dyes to the wastewater. Inflow of coloured wastewaters to the receiver, usually surface waters, is in the great concern, since they reduce light transmission, limit photosynthesis and hence the primary production. Potential toxicity, mutagenicity and carcinogenicity of sewage included dyes and their transformation products has an impact on the reduction of biodiversity of surface water [13-15]. For removal of this type of pollution some of species of fungi, whose ability to degrade the compounds with aromatic structure is well documented, may be used. This group of organisms, as one of the few, is able to break down the complex natural polymer as lignin [1-5]. These unique abilities are associated with the production of enzymes with low specificity, which belong to numerous laccases and peroxidases that are able to break down the lignin aromatic rings [3, 6-10].

Among the fungi producing ligninolytic enzymes should be remarkably distinguished white rot fungi, the most active in the degradation of lignins. One of its representatives is *Hypholoma fasciculare*, classified to the order *Agaricales*. The possibilities of using it in the breakdown of industrial pollution are also favored because of the prevalence and broad coverage of the European and American continents. This fungus is one of the poisonous, what is caused by the highly poisonous fasciculol. Bears fruit from May to November on dead hardwood and softwood [16]. It has been shown that fungi classified in this species among others produce laccases [17-18]. Valášková et al. [19] found that the species *Hypholoma fasciculare* has great potential for degradation of the remains of ligno-cellulose. Within 12 has 23% degraded weeks of the fresh biomass of oak leaves that were colonized fully in just three weeks. At the first incubation period there was observed a significant activity of laccases and a less Mn-depended peroxidases. Despite the high enzymatic activity degradation of lignin was low level. Bending et al. [20] showed that the fungi classified in this species are able to decolorize the polymeric dye Poly R-478 spread added to the medium. According to the authors the process was based on ligninolytic enzymes activities. After the 42 days *H. fasciculare* removed 95% of the dye and was the most effective of all tested species. Used by these researchers strain also showed the ability to degrade a wide variety of pesticides, removing more than 86% terbuthylazine, 55% atrazine and 70% of diuron [20].

Considering the huge degradation potential of representatives of this species the aim of the conducted studies was to evaluate the possibility of use of the strains isolated from two different sites in Upper Silesia in the removal of anthraquinone dye remazol brilliant blue R (RBBR). The impact of biomass immobilization on the effectiveness of the process was evaluated, as well as the toxic impact of dye and their biotransformation products on aquatic organisms which were represented by one consumer (*Daphnia magna*) and producer (*Lemna minor*).

2. MATERIALS AND METHODS

2.1. The biological material

Two strains of *Hypholoma fasciculare* were used in the study. They have been isolated from fruiting bodies collected in Upper Silesia in May (L1) and early autumn (L3). The isolation was done with spore print method and the medium used for spore growth was MEA (Difco). Mycelium cultures were cultivated at 26°C., and pure strains were stored on the same medium at a temperature of 5°C.

2.2. The used dye

In the research an anthraquinone dye RBBR (C.I. 61200) manufactured by Acros Organics was used. It is classified as anionic reactive dye, characterized by the molar mass 626.54 g/mol and the maximum absorbance in the visible light range $\lambda = 593$ nm (UV-VIS, Hitachi 1900).

2.3. Determination of decolorization potential of biomass Hypholoma fasciculare

In order to determine the possibility of using both strains of *Hypholoma fasciculare* for the removal of anthraquinone dye the decolorization tests were conducted on solid and liquid media. In the first case rich in nutrients MEA medium (Difco), on which the fungus has been isolated and cultured and the medium MSB (glucose – 10 g/l ammonium tartrate – 0.2 g/l,

thiamin – 10mg/l, $MgSO_4$ * $7H_2O$ – 0.5 g/l $CaCl_2$ * $2H_2O$ – 0.1 g/l, KH_2PO_4 – 2 g/l agar – 20 g/l; pH 5.6) were used. All substrates were sterilized by autoclaving (121°C, 30 minutes). To the medium was added the aqueous solution of dye RBBR. The dye concentration in sample reached 0.1 g/l. The substrates were poured into Petri dishes having a diameter of 6 cm. The disc of the mycelium of the strain having a diameter of 0.5 cm was placed in the central part of prepared Petri dishes. The sample were prepared in 4 replicates for each strain and each of the substrates. Daily observations of mycelium growth were carried out, colony diameter and the diameter of decolorization zone were noted in each day and factor of decolorization (D) was estimated.

In parallel, confirmation tests of the ability of decolorization of RBBR using PG medium (glucose 5 g/l, peptone 1 g/l, KH_2PO_4 1g/l, $MgSO_4$ 0.5 g/l; pH 5.6) were done. This studies aimed to answer whether tested strains remove dye by adsorbtion or by transformation based on enzymatic processes. To tubes with 10 ml of medium (20 ml tube) mycelial disc (diameter 0.5 cm) cultured on the substrate MEA were added. For each of the strains we prepared 12 repeats, which were incubated 7 days at 25°C. After incubation, four samples were filtered through syringe PVDF filter with a pore 20 μm (Whatman) to separate the biomass hyphae from enzymes produced by the strains. Filter sterilized aqueous solution of RBBR was added to 4 samples without biomass and to the 4 samples with biomass. The final concentration of RBBR was 0.1 g/l. Incubation was carried out for 72 h at 26°C, then in all the samples the absorbance was measured. After completion of the experiment the dry weight of mycelium was evaluated.

2.4. Determination of the effect of mycelium immobilization on the efficiency of the dye decolorization

The effect of immobilization of biomass on the effectiveness of decolorization of RBBR were estimated. The mycelium of both strains in suspension, and the mycelium immobilized on a polypropylene foam were used. Material used as carrier was well colonized by the mycelium, all its fragments were entirely covered with mycelium of both strains The medium used in the studies was PG, which composition is given in Section 2.3. The evaluation of the possibility of RBBR sorption by sponge polypropylene was done. For this purpose, to the flasks containing only the carrier an aqueous solution of RBBR was added. The concentration of the dye in sample was 0.1 g/l. In parallel controls were prepared – first comprising a support (~0.5 g in the form of a square with sides of about $1cm^2$) and water, and second which was the aqueous dye solution. All samples were incubated for 4 h at 26°C and then absorbance of the supernatant was measured at wavelength $\lambda = 593$ nm. The concentration of the dye was read from a standard curve. Adsorption of the dye was determined from the formula:

$$A = (1 - \frac{P}{K}) * 100\% \quad (1)$$

Where:

A – adsorption of dye on carrier [%],

P – concentration of RBBR in a sample after 4h of incubation (after subtraction of background color due to the possible released from material);

K – dye control.

In the main experiment, the supports (carriers) were introduced to the flasks (volume of 250 ml) containing 100 ml of medium PG. The material introduced does not cover more than ¾ volume of the flask, and the weight of the carrier in each flask was identical. In parallel trials containing only growth medium were prepared. Thus prepared materials were autoclaved (121°C, 30 minutes). To samples with a sterile medium inoculated with 1 ml of homogenized mycelium which was previously cultured on the medium PG (6 days). Mycelium homogenization was done using BagMixer 400P (10 min.).

Samples with suspended biomass were incubated in static conditions. In order to enhance the colonization of carrier by mycelium all inoculated flasks were incubated in shaken conditions (100 rpm, 26°C). After 6 days of incubation, sterile solution was introduced to the cultures of RBBR (starting dye concentration in sample was 0.1 g/l). Samples with carrier were incubated on a shaker, and the other under static conditions. The following modifications of trials were prepared for each strain: medium with suspended mycelium and dye (B); medium with mycelium immobilized on support and dye (NB); control with medium and dye (KB); control with the medium and carrier, and dye (NKB); control containing only medium (P); control with medium and support (PN); control with medium and strain (PSZ); controls with medium, carrier and strain (PNSz). All of the sample, including the controls were prepared in four replications. The flasks were incubated for 96 h. For measurement of absorbance and determination of the changes in concentration of the dye over time the

samples were taken at 1, 3, 24, 48, 72 and 96 h of experiment. After the decolourization test, the concentration of dry biomass in the trials was measured. For this purpose the sample was filtered through a cellulose filter, and dried at 55°C to constant weight. For samples with a carrier in the calculation of the concentration of dry biomass weight of the support added to each sample was subtracted.

The degree of decolorization was determined according to formula:

$$U = (1 - \frac{\mathrm{Pr}}{K}) * 100\% \quad (2)$$

Where:

U – removal of dye [%],

Pr – concentration of RBBR in the sample after a given time [g/l]: for samples with suspended biomass Pr = B-PSz, for immobilized biomass Pr = NB-PNSz.

K – adequate control with dye [g/l]: for suspended biomass K = KB-P, for immobilized biomass K = NKB-PN.

2.5. Evaluation of ecotoxicity of the post-process samples

For all samples, including the control samples, toxicity tests were conducted on species of *Cladocera* which was *Daphnia magna* (OECD 202) and the duckweed *Lemna minor* (OECD *Lemna* sp. growth inhibition test. No. 221). Both test oganisms were taken from our own farm. For testing of *Daphnia* individuals aged 48 hours were used. For zootoxicity on each repetition of tests and each dilution 20 individuals were used. The test organisms were introduced into the 5 ml wells of plates. The samples were incubated in the dark at. 21°C. After 24 hours of exposure individuals showing no mobility were counted, on the basis of this results the EC50 was calculated. For phytotoxicity test for each dilution and each repetition 4 specimens with the three fronds were used. Assays were carried out in tubes, in which the organisms were exposed at 24°C in daylight. The parameter measured in this case were any chlorotic and necrotic changes. All tests were performed in 4 replicates for the raw sample and dilute exponentially (from 100% of the sample to 0.78%). We determined EC50 value (concentration causing 50% effect of the test organism), and on the basis of that acute toxicity TUa unit (TUa = 100/EC50), which allowed to determine the class of sample toxicity. According to the ACE 89/2 BE / D3 Final Report Commission EC if TUa < 0.4 means that the sample is not toxic (Class I), if 0.4 ≤ TUa < 1.0 means that the samples is low toxic (Class II) if 1.0 ≤ TUa < 10 – samples are toxic (class III), if 10 ≤ TUa ≤ 100 – samples are highly toxic (class IV), and if TUa > 100 – samples are extremely toxic (class V).

2.6. Statistical analysis

In statistical analysis program Statistica 5.1 was used. Analyzes were performed for the entire cycle of process using ANOVA test NIR. To verify the assumed hypothesis threshold of significance $\alpha = 0.05$ was employed.

3. RESULTS AND DISCUSSION

3.1 Determination of decolorization potential of *Hypholoma fasciculare*

One of the most important test to identify the possible use of fungi strains in the process of decolorization is plate assay in which substrate dye is added to solid. The observed clear zone around the colony indicate the secretion of enzymes to the substrate to allow decomposition of dye. For both tested strains clear zones around the colony were observed (Fig. 1). The largest zone of decolorization was observed on the MEA medium after the first day of inoculation (coefficient of decolorization ~1.23). With the passage of the time index decreases for both strains. In the last day (120h) zone of decolorization was slightly higher than the same colony, which occupied more than 90% of the surface of the plate. At the same time, there was no statistically significant difference between the coefficients obtained for both strains. In the case of the second substrates (MSB), wherein the amount of organic nutrients was lower, there were clear differences in the size of the coefficient. For strain L1 the highest coefficient (1.23) was achieved after 96 h incubation of the strain, when on MEA at the same time, this ratio was only 1.08. In the case of the strain L3 the highest coefficient of decolorization was achieved after 24 hours of incubation and it reached 1.43. The mycelium grew much slower on the MSB than on the MEA medium. In cultures of both strains significant clear zone was obtained after 120 hours of incubation (decolorization coefficient for L1 and L3 respectively reached 1.19 and 1.12). Although the results indicate that a lower concentration of nutrients in the medium MSB can stimulate the production of enzymes required for damage to the structure of the dye, ANOVA statistical analysis showed

that both the composition of medium and the type of strain has no significant effect on the efficiency of the process of decolorization of RBBR on solid medium. On the MEA medium differences in sizes of the clear zones caused by both strains were not statistically significant ($p = 0.687$). More distinct differences were observed between the results obtained for both strains on the medium MSB ($p = 0.168$). The composition of medium may be important for decolorization by the strain L1 ($p = 0.110$), but not for strain L3 ($p = 0.370$).

Studies on the influence of medium composition on the effectiveness of decolorization of dyes had been done previously for the representatives of this species. The same as with strains L1 and L3 for previously studied strain MW143 better results of removal of the tested triphenylmethane dyes were obtained on the medium MSB. For the above-mentioned strain MW143 removal was 86.6% for the brilliant green and 65.0% for gentian violet [21]. Simultaneously, the same strain much better decolorized samples containing azo Evans blue when medium was poorer in nutrients (dye removal 70.6%), on the medium MSB and 34.9% in YEPG medium) [21]. These studies were conducted, however, in liquid media. The influence of medium composition on the effectiveness of decolorization were also studied by Eichlerova et al. [22] who, for strain belonging to the *Pleurotus* species, observed the complete removal of orange G, and RBBR after 12-28 days of incubation of the plates. The efficiency of the process was dependent on the substrate on which they grown the mycelium. Novotny et al. [23] found that some dyes are more effectively removed when in the medium was considerable amount of nitrogen for growth of the mycelium, but in case of other strains dyes removal was more efficient on media where the concentration of the substrates was very limited. Particularly marked differences were observed for decolorization of anthraquinone dyes.

Tests using *H. fasciculare* on solid media with the addition of dyes were conducted by Junghanns et al. [24]. They studied the ability of decolorization of different dyes by a strain UHH 1-4-03 isolated from water samples. This strain actively removed dyes added to solid substrate. At the same time, tests were conducted in a liquid phase, which showed two anthraquinone dyes: RBBR and the acid blue 62, and a diazo-1 direct blue, dye removal respectively 82, 87 and 95% after 7 days of incubation. At the same time they observed a shift in the maximum of absorbance spectrum, which confirmed that the process had the

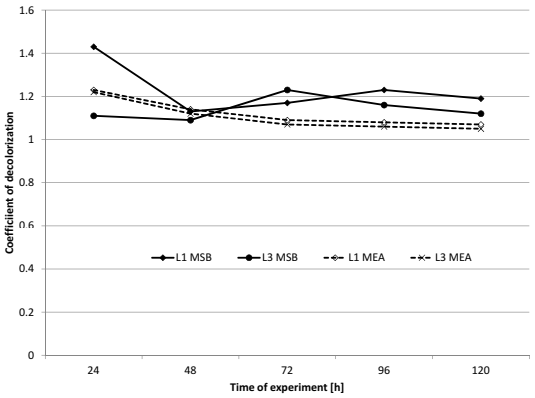

Figure 1.
Changes in the mean values of the coefficient for RBBR decolorization for each strain in the test on solid media

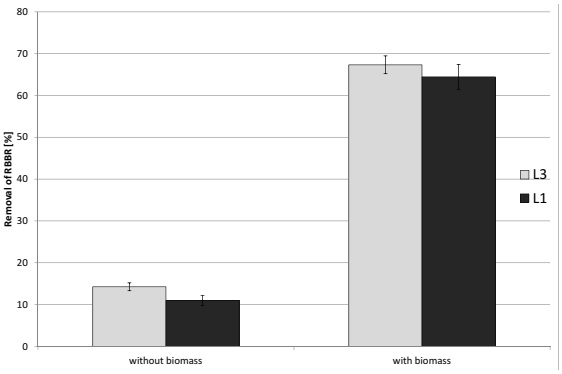

Figure 2.
Removal of RBBR in samples with biomass and without biomass

character of biological decomposition.

For both strains of *H. fasciculare* (L1 and L3) was performed a similar study with RBBR added to the liquid medium. The samples were prepared with biomass and without biomass (Fig. 2). After 72h incubation for samples containing biomass of both strains reduction of color were more than 60%. These results are similar to those obtained by Junghanns et al. [24]. At the same time samples without mycelium, which contained only the enzymes and metabolites produced by mycelium obtained only about 10% loss of color. That indicates the participation in removal of RBBR the enzymatic and adsorption mechanisms (Fig. 2). These results at similar relationship of the lignocellulosic debris observed Valášková et al. [19].

3.2. Determination of the effect of immobilization of mycelium on the efficiency of the dye decolorization

The degree of adsorption of RBBR on a carrier used for the immobilization of biomass was 0.04%, indicating that the carrier was not involved in the process

of removal of dye. The results of decolorization of RBBR by both strains are shown in Fig. 3.

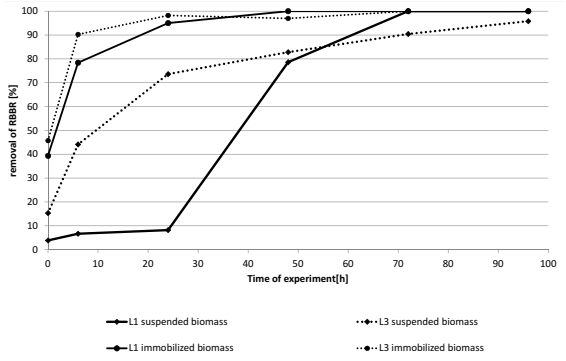

Figure 3.
The efficiency of decolorization of RBBR by immobilized and not immobilized mycelium

Immobilization of biomass contributed to the increase in the degree of decolorization of RBBR already in the first hours of the experiment. After 6 hours from the addition of the dye into samples with suspended biomass of strain L1 reduction of color was less than 7% and remained at a similar level to 24h. In the case of the strain L3 after 6h loss of RBBR was at ~44% and after 24 hours at the level of 73.6%. For samples immobilized the removal for the strains L1 and L3 was about ~40% from the start of the experiment and after 24 hours the removal reached 95% and more than 98% respectively. After 72h of the experiment in trials with immobilized biomass we observed the complete removal of color and this state did not change until the end of the experiment (no desorption). For samples with the biomass suspended in a medium removal after 72 hours reached 90% for the strain L3, while the strain L1 completely removed the dye. The differences between the results for both strains were not statistically significant. ANOVA analysis (Table 1) performed for the whole period of the experiment demonstrated that the results obtained with both immobilized strains were very similar ($p = 0.937$), in the case of static samples did not differ significantly ($p = 0.619$). Much bigger differences were observed in the case of the effect of immobilization of mycelium on the effectiveness of decolorization. For the strain L1 differences in the removal of color between the samples with suspended biomass and samples with biomass immobilized were significant, especially at the beginning of the experiment, however, speaking about the process as a whole they were not statistically significant ($p = 0.278$). Similar differences were also obtained for the strain L3 ($p = 0.348$).

Table 1.
Determined p-value in experiment with suspended and immobilized biomass

	Immobilized biomass	Suspended biomass
Differences in decolorization efficiency between strains L1 and L3	p=0.937	p=0.619
	Strain L1	Strain L3
Differences in decolorization efficiency between biomass immobilized and suspended	p=0.278	p=0.348

The positive influence of immobilization on the efficiency of decolorization was emphasized repeatedly. The main reason for better results are considered to be improved conditions for growth of mycelium, increased enzyme activity or increased contact surface of the mycelium with contamination [5; 25-27]. The results for the both strains suggest the possibility of using both of them for the effective removal of dye from the water. Tychanowicz et al. [28] found that the mycelium of *Pleurotus pulmonaris* immobilized on the cob corn completely removes azo Congo red, trypan blue, ethyl violet and brilliant, methyl green and RBBR within 6 days. In the case of L1 and L3 strains such results were obtained after 3 days. Comparable results were obtained by Siri et al. [29] – the immobilized mycelium of *Trametes versicolor* needed only 3h to remove half of the added to medium RBBR and after 6h the removal of dye was over 85%. Daâssi et al. [30] obtained a 88.7% removal of the dye Lanaset Grey G (LG) after 72h of incubation of mycelium *C. gallica* immobilized in alginate. Immobilization of biomass afforded 75% of discoloration even after three cycles of operation of reactor.

It was found that immobilization contributes to the intensive growth of the mycelium (Fig. 4). In the case of the strain L1 biomass concentration was approximately 20% higher if it was immobilized, and for strain L3 almost doubled. Differences in level of biomass growth stimulation in case of immobilized samples could be connected with specific properties of strains. This had a reflection in results of decolorization achieved during the study, because just for the strain L3 this process was the most effective.

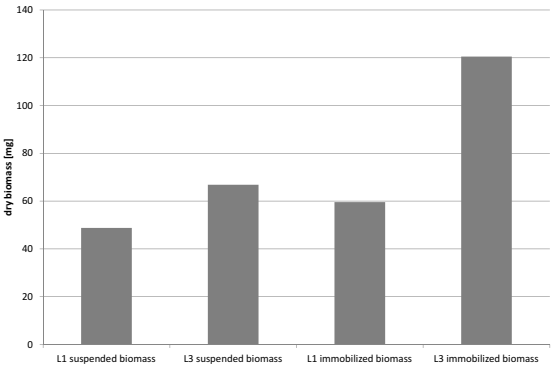

Figure 4.
Dry biomass [mg] of *Hypholoma fasciculare* in different growth conditions

Zootoxicity test for both control of the dye and the samples with mycelium of both strains (immobilized or suspended) allowed to classify the samples to I class of toxicity, what means that samples are not toxic to the tested organism. There was no negative impact of RBBR on test organism, which did not allowed to determine EC50 and TUa (Table 1). In the case of phytotoxicity a negative impact of RBBR on *Lemna* sp. was observed EC50 reached 13.2% for the control with dye, and TUa 7.58, which allowed to qualify this control as toxic. The process of decolorization RBBR had contributed to the elimination of toxicity of RBBR and samples with mycelium of both strains were classified into class I. There was no negative effect of these samples on the growth of *Lemna* sp. EC50 was not determined, therefore, samples were non-toxic.

4. CONCLUSIONS

The efficiency of decolorization was different for the two tested strains and also depends on the composition of the substrate. Slightly larger clear zone reached in the plate test was obtained on MSB medium, much poorer in nutrients than the medium MEA. The differences in the results of decolorization on both media were not statistically significant. In the case of using both strains in liquid culture complete discoloration RBBR was obtained. Thus these studies indicate that the fungi of the species *Hypholoma fasciculare* may be used for decolorization of anthraquinone dyes such as RBBR. The confirmation of this were both obtained the efficiency of decolorization and results of toxicity tests, pointing to decrease of phytotoxicity of samples after decolorization process. Complete removal of color may be obtained if the mycelium is immobilized on a carrier. The carrier in the form of polypropylene foam is rapidly colonized by the mycelium and speeds up the removal of color, what allows to shorten the time of decolorization up to 48-72h. This phenomenon was observed even at a relatively low concentration of biomass noticed for strain L1. Particularly promising results were obtained for the strain L3 for which we observed a doubling of a biomass in immobilized samples as compared to samples with suspended biomass, and after 48 hours of incubation RBBR was completely removed from the medium (samples with immobilized mycelium).

4. SUMMARY

The effectiveness of decolorization of RBBR by two strains was different and depended on the composition of the medium. Slightly larger zone of discoloration in the plate test was obtained for MSB medium, which has lower concentrations of nutrients than the medium MEA. The differences in the results of decolorization on both media were not statistically significant. In the case of using of both strains in liquid culture complete removal of RBBR can be reached. The results thus indicate that the fungi of the species *Hypholoma fasciculare* can be used for decolorization of anthraquinone dye such as RBBR. The confirmation of that statement are the results of efficiency of decolorization and toxicity tests. In after process samples decrease of phytotoxicity was observed. Complete removal of color can be obtained if the mycelium is immobilized on a solid support. Polypropylene foam used as solid support was quickly colonized by strains and accelerates the removal of color, allowing to reduce the time of decolorization up to 48-72h. This phenomenon was observed even at a relatively low concentration of biomass of strain L1. Especially promising results were obtained for the strain L3 for which doubling of biomass concentration was observed in immobilized samples as compared to samples with suspended biomass. That strain after 48 hours of incubation completely removed RBBR from the medium (sample with the immobilized mycelium).

ACKNOWLEDGEMENT

This research has been supported by the BK-271/RIE-8/15.

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