DOI: 10.2428/ecea.2020.27(1-2)2 ECOL CHEM ENG A. 2020;27(1-2)

Wioletta PRZYSTAŚ[1*] and Ewa ZABŁOCKA-GODLEWSKA[1]

# IMPROVEMENT OF EFFICIENCY OF DYE REMOVAL BY IMMOBILIZATION OF FUNGAL BIOMASS

## POPRAWA EFEKTYWNOŚCI USUWANIA BARWNIKÓW POPRZEZ IMMOBILIZACJĘ BIOMASY GRZYBOWEJ

**Abstract:** One of the ways to increase the efficiency of the decolorization process is the immobilization of biomass. In order to improve the efficiency of the dyes removal, a set of tests were carried out. The immobilized mycelium of the following strains: *Pleurotus ostreatus* (BWPH), *Hypholoma fasciculare* (L3) and *Pluteus* sp. (RSW3) were used to remove dyes from different classes (azo, antraquinone and triphenylmethane dyes). Two types of carriers were used – natural and synthetic. It was shown that the immobilization of the mycelium on the carriers allowed for the complete decolorization but not for all dyes. It was noted that the efficiency of the process depended on the strain, dye and carrier used for immobilization. Strain RSW3 (immobilized on pistachio shell) and RWP17 (immobilized on sponge) removed completely the RBBR anthraquinone dye, and almost all Evans blue. In case of brilliant green almost all dye was removed by strain L3 immobilized on pistachio shell.

**Keywords:** fungi, decolorization, azo dyes, anthraquinone dyes, triphenylmethane dyes

## Introduction

The widespread use of dyes in various industries contributes to a significant contamination of the environment with these substances. The danger of dyes penetrating the environment is mainly due to their properties (aromatic structure, persistence). Dyes are produced on a mass scale and are designed to give color to dyed products permanently. In principle, they are substances that are poorly susceptible to UV radiation or microorganisms biodegradation [1, 2]. In addition, a significant part of these substances has been proven to be toxic to many organisms as well as mutagenic or carcinogenic. Despite many years of research, the problem of removing of dyes from industrial wastewater is still a challenge. It is already known that the use of standard activated sludge processes does not effectively eliminate these compounds. Activated sludge mainly absorbs dyes, and such a process may pose a risk of desorption [2–5, 7].

[1] Department of Environmental Biotechnology, Faculty of Energy and Environmental Engineering, Silesian University of Technology, Akademicka 2A, 44-100 Gliwice, Poland, phone: +48 32 237 28 55.

* Corresponding author: wioletta.przystas@polsl.pl

Currently conducted research aimed at eliminating dyes from wastewater concerns the possibility of using many different groups of organisms, including fungi, bacteria and plants. Each of these groups has different properties and may use different removal mechanisms: sorption and/or biotransformation by different enzymes in different conditions. Research with the participation of fungi is of great importance, because the processes of decolorization occurs in their case due to the production of a number of exoenzymes with low specificity (as laccase, Mn-dependent peroxidase, versatile peroxidase or lignin peroxidase). Both the biomass itself, which also sorbs dyes, as well as the extracted enzymes can be used. It should be remembered that use of extracted enzymes is expensive. The main advantages of using biological processes are their economy and environmental friendliness. The cultivation of biomass with appropriate properties, especially in the case of using waste materials as a source of carbon and energy, is a cheaper process than the use of physicochemical techniques in removing pollutants [2, 5, 6, 8–10]. Biomass immobilization in order to accelerate the processes has been widely used in biotechnology for many years. An example is wastewater treatment with the use of a biological bed, especially in the removal of industrial wastewater. Immobilization is used to increase the production of enzymes or to protect the microorganisms applied in the processes against the harmful effects of toxic compounds. Of course, the immobilization process can also apply to the enzymes themselves. However, such an application does not significantly reduce the cost of the decolorization process and is not a much more interesting alternative than the use of chemical or physical processes in decolorization [8–10].

The aim of the research was to evaluate the effect of mycelium immobilization on the efficiency of decolorization and detoxification of dyes of various classes. For this purpose, Evans blue, brilliant green and remazol brilliant blue R were used, and three strains of Basidiomycota representing different species.

## Materials and methods

### Strains of fungi

All the strains used in the research come from the collection of the Department of Environmental Biotechnology of the Silesian University of Technology. The strains belong to the species: *Hypholoma fasciculare* (strain L3), *Polyporus picipes* (strain RWP17) – a species that has not been studied well in terms of decolorization potential, and the last strain belongs to the genus *Pluteus* (strain RSW3). The strains L3 and RSW3 were isolated by the tissue method from fruiting bodies collected in Upper Silesia, and the strain RSW3 from spores. The mycelium of all strains was grown and stored in Difco's MEA medium.

### Dyes and other materials

The dyes used in the research belonged to the most commonly used classes: azo Evans blue ((BE) manufacturer Sigma-Aldrich), triphenylmethane brilliant green ((ZB) manufacturer Sigma-Aldrich) and anthraquinone remazole brilliant blue R ((RBBR)

manufacturer Acros Organics). Research with the use of these dyes has already been conducted and presented [6, 11]. Aqueous dye solutions were prepared and mechanically sterilized using syringe filters with a pore diameter of 0.2 μm. Such sterile solutions were introduced into the research trials. The initial concentration of dyes used in the tests was 0.1 g/dm$^3$.

The research was carried out on a liquid PG medium with the following composition: glucose 5 g/dm$^3$, peptone 1 g/dm$^3$, $KH_2PO_4$ 0.1 g/dm$^3$, 0.5 g/dm$^3$ $MgSO_4 \cdot 7H_2O$ and pH = 5.6.

The research was carried out with the use of two carriers – natural and synthetic. On the basis of previous tests [11], a polyurethane sponge (for dish washing) was selected. It is an artificial material, which is characterized by a very porous structure favoring the growth of mycelium. A waste material in the form of pistachio nut shells was used as a natural carrier material. Before being used in the research, the polyurethane material was rinsed: 4 cycles of 15 minutes each with distilled water in order to eliminate possible chemical contamination. Fragments with a side of 1 cm were used in the research. Pistachio shells were used unchanged, in the form of their halves.

The culture medium with the carrier fragments was sterilized in an autoclave (121 $^oC$, 30 min, 147 kPa) before inoculation with mycelium.

## Influence of the type of carrier on the efficiency of dyes removal

The research on the removal of dyes from three different classes was carried out on the PG medium in modifications: mycelium immobilized on polyurethane material, mycelium immobilized on pistachio shells, mycelium suspended in the medium. Appropriate controls were prepared for each modification: 1). controls containing culture medium and dyes without carrier, 2). controls containing medium, carrier and dye, 3). controls containing medium and carrier without dye, 4). controls containing medium, 5). controls containing medium and mycelium of a given strain, 6). controls containing medium, mycelium of a given strain and carrier. All modifications and controls were set up in 4 replicates. The tests were carried out in flasks containing 80 cm$^3$ of sterile PG medium and carriers: 1 g of a polyurethane sponge cut into a 1 cm side fragment or 10 g of pistachio shells. The homogenized suspension of the hyphae of the strains (1 cm$^3$) was introduced into the thus prepared sterile samples. Before the dyes were added, the mycelium was incubated on a shaker (26 $^oC$ and 150 rpm) for a period of 7 days. The sterile dye solutions were introduced in such a way that the initial concentration in the test was 0.1 g/dm$^3$. The absorbance was measured by taking 2 cm$^3$ of the sample solution after: 1, 4, 24, 48, 72 and 96 h. Analyses were made using a UV-Vis Hitachi U-1900 spectrophotometer, and the removal value at a given time was calculated from the formula:

$$R = \frac{(C - S)}{C} \cdot 100 \quad [\%]$$

where $R$ is the dye removal after a given time, determined as a percentage, $C$ is the concentration of dyes in the control sample [g/dm$^3$] after a given time and taking into

account the color given to the solution by an appropriate carrier, *S* is the dye concentration [g/dm$^3$] after a given time (also taking into account any possible colour changes caused by mycelium).

After the experiment was completed, the dry biomass content in the samples was also measured. For this purpose, the samples, after the end of the tests, were filtered through medium filters and dried at the temperature of 55 $^o$C until constant weight was reached. Measurement of the concentration of biomass and carrier was determined by the gravimetric method.

## Results and discussion

Effective removal of dyes from waste water can be achieved through the use of physical and chemical processes. The dye adsorption processes have received the most attention. By using activated carbon and natural sorbents, significant amounts of dyes can be removed. However, the process depends on many factors, not only the active surface of a given sorbent, but also the type of dye and its concentration [12]. It seems, therefore, that the combination of sorption process with biodegradation is one of the best ways to improve the effectiveness of decolourisation. In this study we adopted a strategy to link the two processes. Two types of carriers (natural and artificial) were used to better assess the possibilities of using selected fungal strains to remove dyes belonging to different classes.

The research includes the comparison of the effectiveness of the decolourisation process by the mycelium growing on two types of carriers and in the form suspended in a liquid medium. The removal of individual dyes by the carriers themselves was also analysed. The test results are presented in Figures 1–3.

In the case of Evans blue (Fig. 1a–c), the degree of dye adsorption on the polyurethane sponge increased slowly during the 96 hours of the experiment. Finally, the sponge removed the dye in 12.2 %. Natural material in the form of pistachio shells, despite its relatively low porosity, removed this dye at the same time in 32 %. Natural materials are known for their high sorption properties [6, 7, 12, 13].

Much better results of Evans blue removal were obtained for the mycelium immobilized on the carriers. The highest degree of decolorization was obtained for the RSW3 strain immobilized on pistachio shells (95 % Fig. 1b) and for the RWP17 strain immobilized on a sponge (93.5 % after 96 h – Fig. 1c). For both strains, the removal by non-immobilized mycelium was 19.5 % and 73.3 %, respectively. The efficiency of decolorization by the L3 strain immobilized on the shells was also high and finally reached 90 %, while the mycelium suspended in the medium removed 61.7 % (Fig. 1a). The obtained results indicate that the influence of the carrier used for immobilization on the intensity of the Evans blue removal process is not clear. Both, in the case of strain RWP17 immobilized on shell and mycelium of L3 strain immobilized on a sponge, the differences were small. This indicates the lack of one universal mycelium carrier and the need for individual selection of the carrier for a specific strain in order to optimize the dye removal process. The results of biomass concentration in individual samples (Table 1) indicated that the decolorization process does not depend on its concentration.

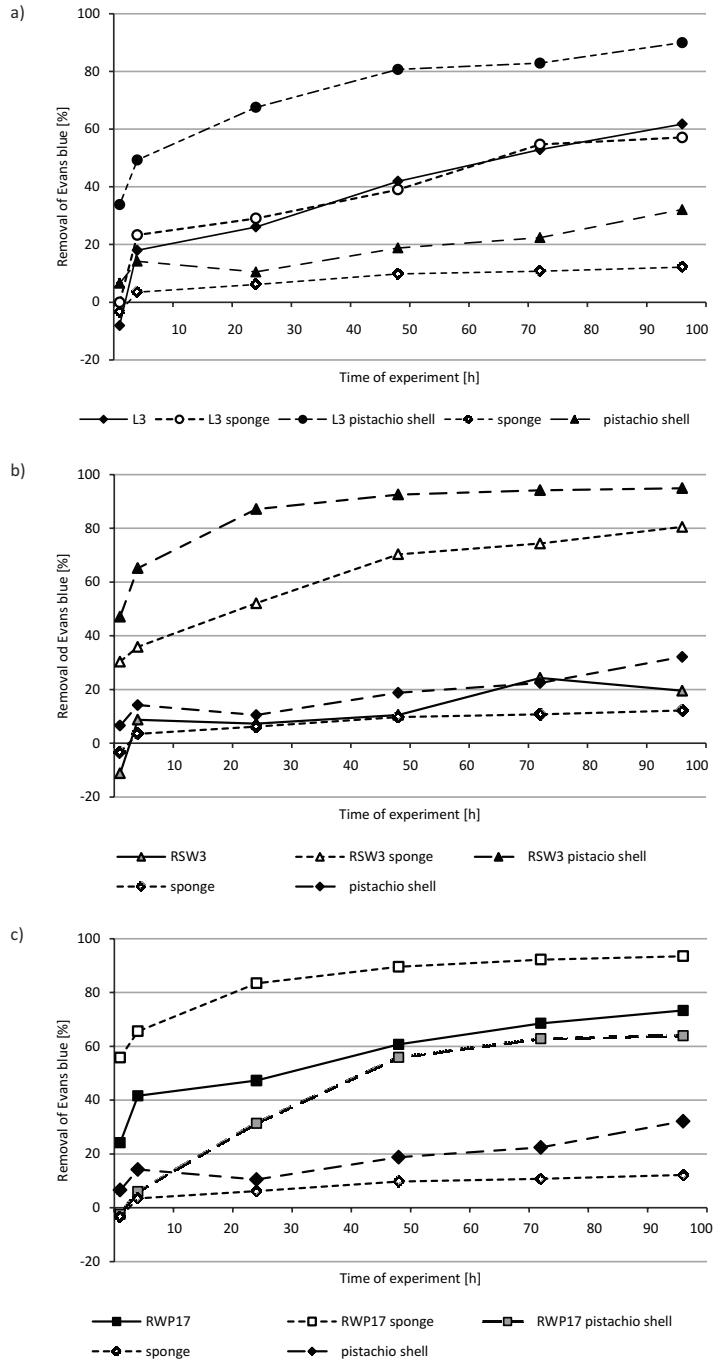


Fig. 1. Percentage removal of Evans blue in samples with different strains and carriers used for immobilization: a) strain L3, b) strain RSW3, c) strain RWP17

The highest biomass concentration was obtained in samples with the mycelium of strain RSW3 but in these samples the removal of Evans blue was low (< 20 %). The lowest concentration of mycelium was in the non-immobilized samples for the L3 strain, which removed over 60 % of the dye.

Table 1

Concentration of dry biomass of mycelium in samples with Evans blue (at the end of experiment)

| Strain | Concentration of dry biomass in sample with medium [g/sample] | Concentration of dry biomass in sample with polypropylene sponge [g/sample] | Concentration of dry biomass in sample with pistachio shells [g/sample] |
|---|---|---|---|
| L3 | 0.03 | 0.11 | –1.01 |
| RSW3 | 0.08 | 0.18 | –0.90 |
| RWP17 | 0.04 | 0.08 | –1.10 |

The positive effect of immobilization on the removal of various contaminants has been emphasized many times [5, 6, 10, 14]. The high efficiency of Evans blue decolorization by the immobilized mycelium of the RWP17 strain was demonstrated, the same as it was also noted that the carrier used for immobilization is of great importance [6].

In contrast to azo Evans blue, triphenylmethane brilliant green was strongly absorbed by each of the materials used for immobilization (Figs. 2a–c). The synthetic material in the form of a sponge absorbed 78 % of the dye added to the substrate within 96 hours, and the natural material in the form of pistachio shells – 95.7 %. While the results obtained for the natural material are not surprising, in the case of the low porosity synthetic material, the high degree of removal of this dye is surprising. Therefore, compared to Evans azo blue (Fig. 1a–c), this dye seems to be easier to remove and it is enough to use the sorption process in decolorization. In most cases, the degree of dye adsorption on synthetic materials does not exceed 10 % [6]. However, there are few reports on the possibility of using synthetic materials to remove dyes [7].

Taking into account the results of the above-mentioned control samples, the immobilization of the mycelium used for the decolorization of brilliant green did not significantly increase the efficiency of the process (Fig. 1a–c). The difference in the degree of decolorization between the immobilized and suspended mycelium of the L3 strain (Fig. 2a) after 96 h of the experiment was about 10 % in favor of the mycelium immobilized on the support (96.1 % removal), and for the sponge it was about 4 % lower than the non-immobilized mycelium (82.2 % and 86.3 % removal respectively). A slight difference in the final green removal obtained in the control with shells and sample with mycelium would indicate that the process is primarily based on sorption. However, biochemical changes of this dye in samples with mycelium cannot be excluded. As can be seen, the samples with mycelium and shells showed the loss of biomass, indicating the biological decomposition of the carrier by the mycelium (Table 2). The same situation was noticed for other dyes (Table 1 and 3). Moreover, in the first hours of the experiment, the efficiency of the brilliant green removal process in

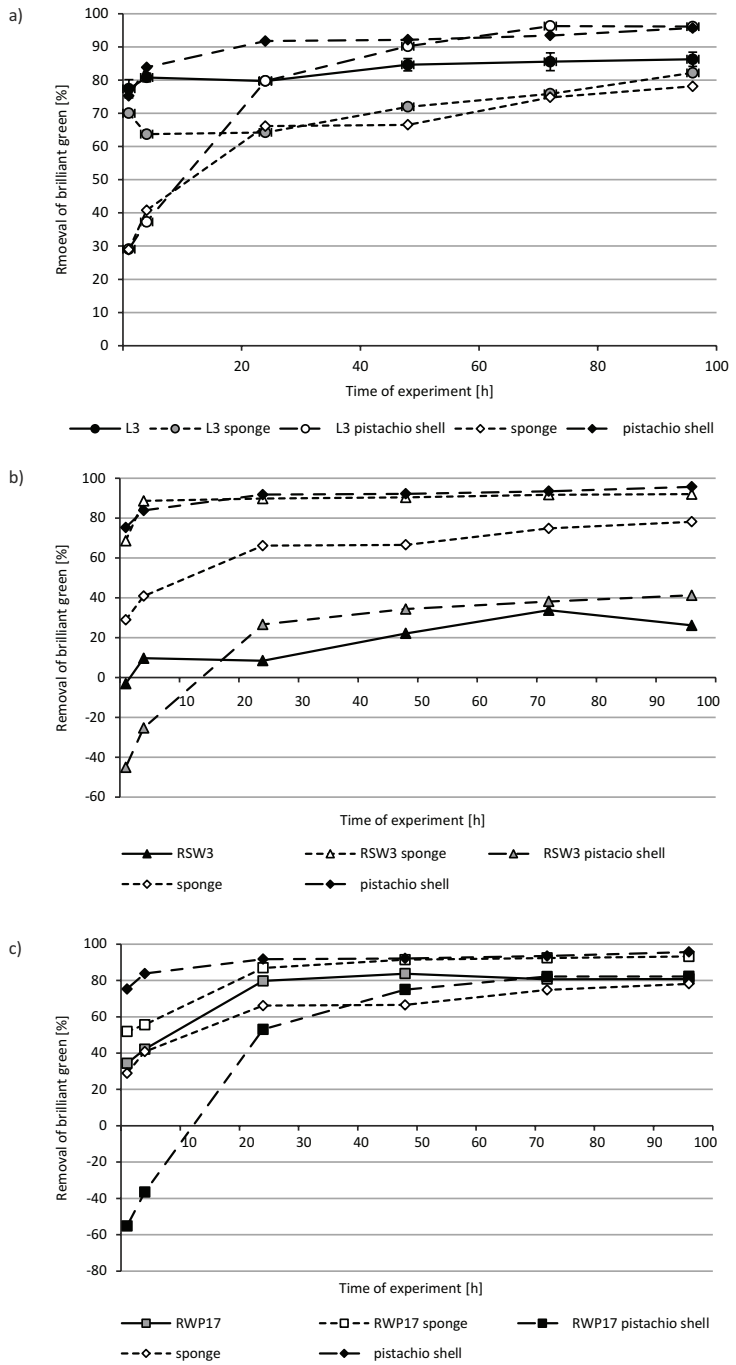


Fig. 2. Percentage removal of brilliant green in samples with different strains and carriers used for immobilization: a) strain L3, b) strain RSW3, c) strain RWP17

the samples pistachio shells with mycelium was 46.2 % lower than in the control with shells (Fig. 2a), therefore the properties of the shells had to be modified, which deteriorated the ability to adsorb this dye on them. Visual observations confirmed the strong colonization of the carrier by the mycelium, which, by decomposing it, intensively changed its properties. However, it is difficult to estimate the share of sorption and biotransformation in the decolorization process for this type of test. In the case of the synthetic carrier (sponge), no significant differences were observed in the effectiveness of decolorization by the carrier itself and the mycelium immobilized on it (removal after 96 h (removal of 78.1 and 82.2 %, respectively)).

Table 2

Concentration of dry biomass of mycelium in samples with brilliant green (at the end of experiment)

| Strain | Concentration of dry biomass in sample with medium [g/sample] | Concentration of dry biomass in sample with polypropylene sponge [g/sample] | Concentration of dry biomass in sample with pistachio shells [g/sample] |
|---|---|---|---|
| L3 | 0.04 | 0.11 | –0.46 |
| RSW3 | 0.15 | 0.10 | –0.12 |
| RWP17 | 0.05 | 0.08 | –0.03 |

Similar relationships were also obtained for the strain RWP17 (Fig. 2c). At the end of the decolorization process, the difference in removal between the controls with the carriers, the non-immobilized mycelium and the mycelium immobilized on both carriers did not exceed 13 %. The greatest removal of brilliant green by the RWP17 strain was obtained in the case of mycelium immobilized on a sponge (93.2 %), and the lowest in the case of mycelium suspended in the substrate (80.8 %).

Completely different results were obtained for the RSW3 strain (Fig. 2b). The non-immobilized mycelium removed after 96 hours only 26.1 % of brilliant green, what is less than the control. Biomass immobilized on the shells removed less than control, because 41.2 % compared to 96 % by the shells in medium. The mycelium immobilized on the sponge removed 92 %, what is 14 % more than the sponge itself. In this case, there is a positive effect of immobilization on the decolorization, but its efficiency depends on the selected carrier. Poor removal when shells are used as a carrier may be connected with strong degradation of the carrier, as indicated by the measurements of dry matter (Table 2). The negative values of the dry matter of the mycelium and the strong growth observed on the support indicate that the shells were partially degraded, which reduced their sorption capacity towards brilliant green. This phenomenon is quite common when using natural biomass carriers [5].

In the case of remazole brilliant blue R, no intensive adsorption was observed on the carriers used for immobilization (Figs. 3a–c). For the sponge, the removal level after 96 hours was only 1.6 % and for the shells 6.11 %. However, the results of decolorization obtained for individual strains were very diverse. The suspended mycelium of strain L3 strain (Fig. 3a), in the medium did not remove this dye, because after the initial adsorption of the dye at the level of 40 %, after 1 h it was desorbed and finally the

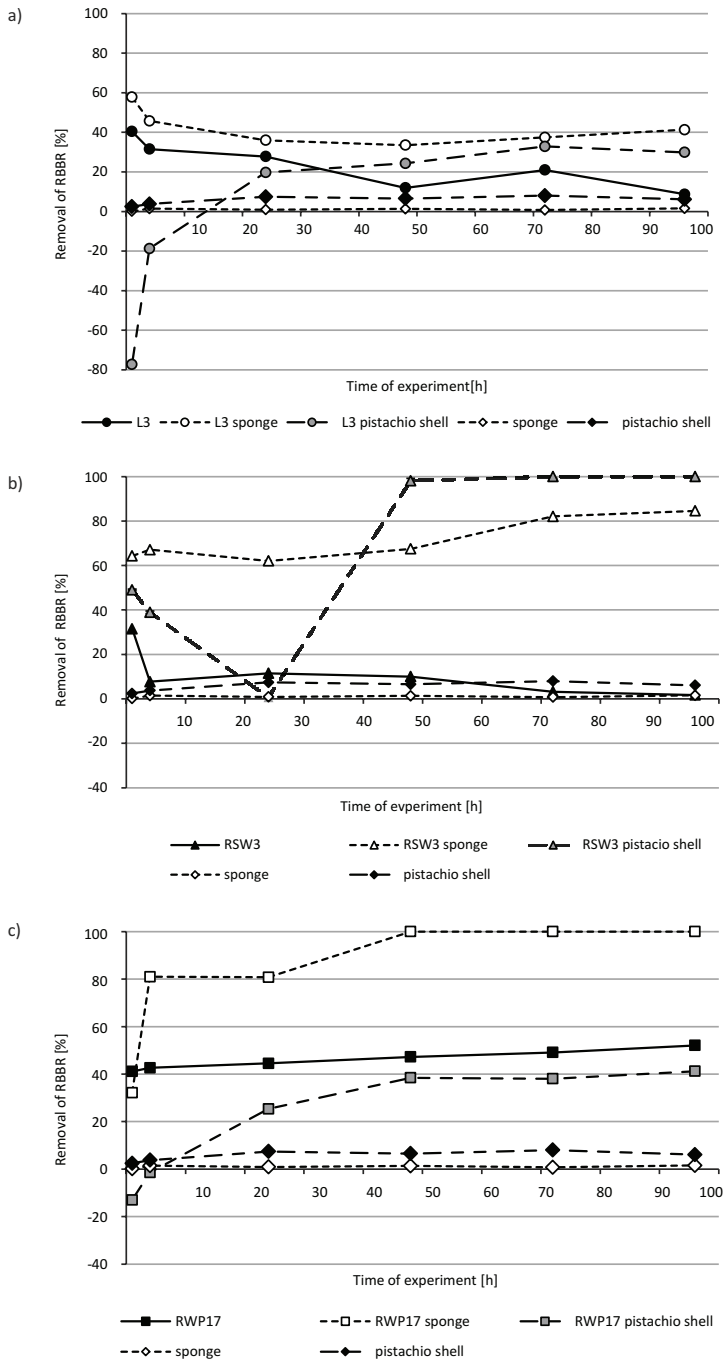


Fig. 3. Percentage removal of RBBR in samples with different strains and carriers used for immobilization: a) strain L3, b) strain RSW3, c) strain RWP17

decolorization efficiency was only 8.7 % of RBBR. The immobilized mycelium of the L3 strain removed 41.3 and 29.9 % (carries sponge and shells, respectively).

This dye was also poorly removed by the RSW3 strain (Fig. 2b) if the mycelium was not immobilized on the support (1.7 % removal after 96 h). Again, after the initial sorption (31.6 % after 1 h), the dye was released from the mycelium suspended in the medium. However, in the case of the sponge as the biomass carrier, 84.6 % of RBBR was removed after 96 h and the process was stable. For pistachio shells, removal was as high as 100 % after 72 hours, but initially the process was unstable. High results of RBBR removal by this strain in 1 h of the experiment (all samples with strain > 30 %) indicate a significant share of sorption in the process, especially in its initial phase. However, the high degree of removal at the end of the study is likely due to biochemical processes involved in later steps in the process.

A positive effect of immobilization on a sponge on the removal of remazole blue was observed for the strain RWP17 (Fig. 3c). Full decolorization was achieved after 48 hours of the process and no fluctuations in the dye concentration in the samples were observed. This indicate that sorption is not the dominant process in the removal by this strain. For comparison, after 96 h the mycelium suspended in the medium removed 52.1 %, and the mycelium immobilized on the shells only 41.2 %.

The test results obtained for RBBR, similarly to the other two dyes, confirm that the degree of decolorization is not dependent on the biomass concentration. The highest biomass was obtained for the RSW3 strain (Table 3) in the sample with the suspended hyphae and in the sample with the mycelium immobilized on the sponge (in both cases 0.13 g/sample), and the highest degree of removal was obtained in the samples with the RWP17 mycelium immobilized on the sponge and with the mycelium RSW3 immobilized on shells (full decolorization after 72 hours). The lack of correlation between the degree of decolorization and the concentration of biomass was previously observed both in the case of fungi [11, 15].

Table 3

Concentration of dry biomass of mycelium in samples with RBBR (at the end of experiment)

| Strain | Concentration of dry biomass in sample with medium [g/sample] | Concentration of dry biomass in sample with polypropylene sponge [g/sample] | Concentration of dry biomass in sample with pistachio shells [g/sample] |
|---|---|---|---|
| L3 | 0.04 | 0.06 | –0.79 |
| RSW3 | 0.13 | 0.13 | –0.69 |
| RWP17 | 0.04 | 0.10 | –0.70 |

## Summary and conclusion

The conducted research confirms that the effectiveness of decolorization depends on the dye, the strain and form in which it is used in process. Complete decolorization was achieved only in the case of the RBBR. Results reached for this dye confirms that the immobilization of the mycelium was of significant importance. Complete removal was

noticed after 48 h in samples with RWP17 strain, and after 72 h in samples with RSW3 strain. However, the carrier on which the mycelium was immobilized was important. In the case of the RWP17 strain, it was a synthetic sponge, and in the case of the RSW3 strain, natural pistachio shells. Evans blue, the azo dye, was removed easily also by immobilized biomass (93.5 % and 95 %, respectively, for the RWP17 and RSW3 strains after 96 h). On the other hand, brilliant green, easily absorbed by both carriers, was better removed in samples with the strains RWP17 and RSW3 immobilized on a sponge (~92 %) and the strain L3 immobilized on pistachio shells (96.1 %). In the case of this dye, removal above 80 % was also obtained with the use of the mycelium of the L3 and RWP17 strains without the use of carriers. Therefore, it was confirmed that in order to optimize the decolorization process with the use of fungi, it was necessary to select properly the process parameters and to analyse whether and what carrier to use for immobilization for each of the dyes. The promising results were obtained for all strains, but the best for two – RSW3 and RWP17. The appropriate selection of the carrier for the immobilization made it possible to remove each of the dyes used at more than 90 %.

## Acknowledgement

This research was supported by the 08/080/BK_20/0075 (BK-216/RIE8/2020) project

## References

[1] Kandelbauer A, Guebitz GM. Bioremediation for the Decolorization of Textile Dyes – A Review. Chapter 26. In: Lichtfouse E, Schwarzbauer J, Robert D, editors. Environmental Chemistry. Berlin Heidelberg: Springer-Verlag; 2005. p. 269-88. ISBN: 978-3-540-26531-3. DOI: 10.1007/3-540-26531-7_26.
[2] Bhatia D, Sharma NR, Singh J, Kanwar RS. Crit Rev Environ Sci Technol. 2017;47(19):1836-76. DOI: 10.1080/10643389.2017.1393263.
[3] Selvakumar S, Manivasagan R, Chinnappan K. 3 Biotech. 2013;3(1):71-9. DOI: 10.1007/s13205-012-0073-5.
[4] Singh L. J Appl Biotechnol Bioeng. 2017;3(5):430-5. DOI: 10.15406/jabb.2017.03.00081.
[5] Rodríguez Couto S. Biotechnol Adv. 2009;27:227-35. DOI: 10.1016/j.biotechadv.2008.12.001.
[6] Przystaś W, Zabłocka-Godlewska E, Grabińska-Sota E. Braz J Microbiol. 2018;49(2):285-95. DOI: 10.1016/J.BJM.2017.06.010.
[7] Panić VV, Šešlija SI , Nešić AR , Veličković SJ. Hemijska Industrija. Chem Ind. 2013;67(6):881-900. DOI: 10.2298/HEMIND121203020P.
[8] Dzionek A, Wojcieszyńska D, Guzik U. Electron J Biotechnol. 2016;23:28-36. DOI: 10.1016/j.ejbt.2016.07.003.
[9] Senko O, Gladchenko M, Maslova O, Efremenko E. Catalysts. 2019;9(4):326. DOI: 10.3390/catal9040326.
[10] Krastanov A, Koleva R, Alexieva Z, Stoilova I. Biotechnol Biotechnol Equip. 2013;27(6):4263-8. DOI: 10.5504/BBEQ.2013.0096.
[11] Przystaś W, Zabłocka-Godlewska E, Grabińska-Sota E**.** Desalin Water Treat. 2019;376-86. DOI: 10.5004/dwt.2019.24314161.
[12] Bharathi KS, Ramesh ST. Appl Water Sci. 2013;3(4):773-90. DOI: 10.1007/s13201-013-0117-y.
[13] Kyzas GZ, Christodoulou E, Bikiaris DN. Processes. 2018;6(9):166. DOI:10.3390/pr6090166.
[14] Partovinia A, Rasekh B. Crit Rev Environ Sci Technol. 2018;48(1):1-38. DOI: 10.1080/10643389.2018.1439652.
[15] Przystaś W, Zabłocka-Godlewska E. Ecol Chem Eng A. 2019;26(1-2):99-112. DOI: 10.2428/ecea.2019.26(1-2)10.