DOI: 10.2428/ecea.2019.26(1-2)10 ECOL CHEM ENG A. 2019;26(1–2):99–112

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MIXTURE OF DYES – STUDY OF ZOOTOXICITY AND REMOVAL BY SELECTED FUNGAL STRAINS

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Abstract: Synthetic dyes, due to their toxicity and low biodegradability, belong to hazardous and hardly removable contaminants present in environment. Even bigger problems are caused by their mixtures. The aim of the study was the analysis of caused by mixtures of dyes (dyes from various classes) for aquatic organisms and the effectiveness of mixtures removal by strains of basidiomycota fungi. Two-component mixtures consisting of anthraquinone remazol brilliant blue R (RBBR), azo Congo red and triphenylmethane brilliant green (concentration range of mixtures 0.01–0.15 g/dm^3) were tested. Zootoxicity was tested on *Daphnia* magna. Three strains of fungi were used: strains BWPH and K4 belonging to the *Pleurotus ostreatus* species and RWP17 belonging to the *Polyporus picipes* species. Removal of dyes was analyzed at the wavelengths corresponding to the highest absorbance.

It was found that mixtures of dyes are much more toxic to the test organism than single dyes. In decolorization studies with increasing of the concentration of mixture 1 (CK+RBBR), increased removal of colour by BWPH and K4 strains was observed. This mixture was well removed by the RWP17 strain and decolorization efficiency decreased with increasing concentration (from 95 % to 75 %). Removal of mixture 2 (CK + ZB) was very high for *Pleurotus* strains – increase of the dye concentration caused a decrease in the efficiency of the process (from 100 % to 0%).

Studies have showed that the presence of dye mixtures in water is dangerous for organisms and the removal of them is very challenging. Efficiency of the process depends on the mixture composition, concentration and the strain used.

Keywords: mixture of dyes, zootoxicity, fungi, decolorization, azo dyes, triphenylmethane dyes, antraquinone dyes.

Introduction

Synthetic dyes, with the aromatic structure, are one of the most important and commonly occurring surface water pollutants. This is due to their widespread use in various industries, including food and textile, cosmetics and many others [1–5]. Especially in the production of textiles and food they are used to give an intense colour, which by

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definition should be resistant to light, microorganisms and in case of clouthing resistant to sweat. This assumption causes the chemical industry to produce dyes that are by definition poorly susceptible to biodegradation. It is also known that many of these compounds are highly toxic. As toxic substances, often with carcinogenic or mutagenic properties, they pose a great threat to ecosystems, especially aquatic ones [4–6].

Due to the aromatic structure and the toxic properties mentioned above, conventional biological wastewater treatment systems, based on activated sludge, are not able to effectively remove most of the dyes. Therefore, large-scale research at the removal of dyes from wastewater focuses on the use of physical and chemical processes, including the combination of biological processes with physical and chemical through the selection of organisms in terms of their ability to remove these substances [7–12].

Bacteria and fungi will be mentioned among organisms with high potential for dye removal. The great importance of fungi in processes of removal of these substances is related to the ability of these organisms to produce low-specific enzymes with a broad spectrum of activity. Such fungi include the group of white rot fungi, producing peroxidases and laccases, involved in the decomposition of polymers such as lignin. It has been proven many times that these organisms can sorb or break down dyes with high efficiency [1, 13–18]. Most current research is mainly focused on the removal of individual substances, including the most commonly used azo dyes. The second group of compounds, the use of which is constantly growing, are triphenylmethane dyes, and the third is anthraquinone dyes [18, 19]. This approach gives us information about the potential of the organisms, however, it has its drawbacks. Dyes in wastewater are mainly found in mixtures. Most often, already on the production plant, sewage containing dyes are periodically deposited in tanks before they are directed to a wastewater treatment plant. The formation of dye mixtures can significantly hinder the process of removing colour, because these compounds can react with each other. Therefore, it should be remembered that even if single dyes are used, mixtures of them are most often introduced into the environment with wastewater [20].

The aim of the research is to determine whether mixtures of dyes are more dangerous to aquatic organisms than single substances, as well as the assessment of the possibility of using selected strains of fungi, which have the ability to remove aromatic dyes from various classes, to decolorize these mixtures.

Materials and methods

Zootoxicity test

Zootoxicity tests were carried out on the crustacean *Daphnia magna*. Acute toxicity test of aqueous solutions of dyes (Congo ed, Remazol Brilliant Blue R and brilliant green) and mixtures (mixtures 1, 2) were performed (OECD Test No. 202: *Daphnia* sp. Acute Immobilization Test). The dye / mixture concentrations used in the test were 0.001 g/dm^3, 0.002 g/dm^3, 0.004 g/dm^3, 0.006 g/dm^3, 0.008 g/dm^3, 0.01 g/dm^3, 0.03 g/dm^3, 0.06 g/dm^3, 0.09 g/dm^3, 0.1 g/dm^3, 0.12 g/dm^3, 0.15 g/dm^3. All toxicity tests were conducted four times.

Observations of test organisms immobilization were carried out after 48 hours. The obtained results are presented in graphs, based on which EC_{50} (50 % effective concentration) values of individual dyes and mixtures were determined with interpolation method. Due to the EU-Directive 93/67/ EEC (Commission of the European Communities, 1996) [21] substances and mixtures were classifies into different classes according to their EC_{50} values: <1 mg/dm^3 – very toxic, 1–10 mg/dm^3 – toxic, 10–100 mg/dm^3 – harmful to aquatic organisms, >100 mg/dm^3 – not classified.

Strains of fungi

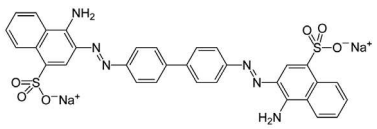
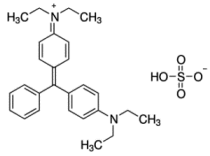
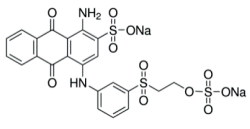
The research was carried out using three strains of fungi for which the ability to decolorize a number of substances had previously been confirmed. BWPH and K4 strains (both belong to the species *Pleurotus ostreatus*) and strain RWP17 (belongs to the species *Polyporus picipes*), all of them come from the collection of the Department of Environmental Biotechnology of the Silesian University of Technology.

Dyes and dye mixtures

To prepare mixtures, dyes belonging to three different classes were used: Congo red (CK) classified as azo dyes, having in its structure two azo bonds; brilliant green (ZB) classified as triphenylmethane dyes; anthraquinone remazol brilliant blue R (RBBR). Characteristic of dyes is presented in Table 1.

Table 1

Characteristics of the dyes used in experiments

Class of dye	Name of dye (manufacturer)	Chemical structure	Chemical structure and molecular weight	λ_{max} [nm]*
Azo	Congo Red (Sigma-Aldrich)		$C_{32}H_{22}N_6Na_2O_6S_2$ 696.67 g/mol	490
Thiphe-nyl-methane	Brilliant Green (Sigma-Aldrich)		$C_{27}H_{34}N_2O_4S$ 482.63 g/mol	624
Antra-quinone	Remazol Brilliant Blue R (Acros Organics)		$C_{22}H_{16}N_2Na_2O_{11}S_3$ 626.54 g/mol	593

* Spectrophotometrically determined wavelength – UV-VIS Hitachi 9000.

Three two-component dye mixtures were prepared in a 1:1 weight ratio. Mixture 1 (M1) consisted of Congo red and RBBR, mixture 2 (M2) consisting of Congo red and brilliant green, and mixture 3 (M3) consisting of brilliant green and RBBR. The last mixture was eliminated from further studies, because both dyes reacted strongly with each other. Rapid precipitation was observed, which could hinder the decolorization tests and measurements of its results. The aqueous solutions of each dye were autoclaved (121 °C, 1.5 atm, 15 min) and the spectra of the solutions prepared in this way across the full wavelength range were checked to eliminate the effect of temperature on the dye structure. Mixtures were prepared and the wavelength (UV-Vis Hitachi 9000 spectrophotometer) at which the highest absorbance was observed was again determined for each mixture. The wavelength values at which the highest peaks were observed are shown in Table 2.

Table 2

Characteristic of mixtures

Mixture	Mixture ingredients	λ_{max} [nm]*
Mixture 1 (M1)	Congo Red + RBBR	228 340 499
Mixture 2 (M2)	Congo Red + Brilliant Green	322 436 630

Studies on the decolorization of dye mixtures by mycelium

Decolorization tests were carried out in tubes containing 10 ml of sterile liquid culture medium composed of: glucose (10 g/dm^3), peptone (1 g/dm^3), $MgSO_4 \cdot 7\ H_2O$ (0.5 g/dm^3) and KH_2PO_4 (0.1 g/dm^3). The medium was sterilized by autoclaving (121 °C, 1.5 atm, 15 min). The sterile medium was inoculated with mycelium of the strains by introducing into the medium 1 ml of mycelium suspension previously grown on the same medium and then homogenized (BagMixer – homogenization time 5 minutes). Mycelial pre-culture at 25 °C lasted 7 days for BWPH and RWP17 strains, and 10 days for K4 strain. Cultivation of the biomass used for decolorization in the tubes was carried out for 7 days at 25 °C. Mixtures of dyes were added to the strain culture in concentrations: 0.01 g/dm^3, 0.03 g/dm^3, 0.06 g/dm^3, 0.09 g/dm^3, 0.1 g/dm^3, 0.12 g/dm^3 and 0.15 g/dm^3. Each sample was prepared in 4 replicates. The same number of control replicates were prepared in parallel: medium with mycelium without dyes, medium with mixtures without biomass. After 7 days of incubation, 1 cm^3 samples were taken from each sample and diluted for UV-VIS spectra. The spectra were analyzed at the characteristic wavelengths of the mixture and the percentage degree of decolorization, R [%] was calculated:

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

where: C – average concentration of the mixture in the control determined for samples containing the mixture in a certain concentration at a given wavelength (mixture control) [g/dm^3];
S – average concentration of the dye mixture after decolorization in a sample inoculated with a given mycelium at a given wavelength, [g/dm^3],

At the end of the experiment, the biomass content of the samples was determined: the biomass was transferred to a paper filter and dried in an oven at 55 °C until they are completely dry.

Results and discussion

Various dyes used at production processes, together with post-process waters, are often periodically deposited in retention tanks before they reach the wastewater treatment plant. This caused the formation of mixtures of unknown composition and properties, both physico-chemical and toxic. As part of the research, several different two-component dye mixtures were prepared. The composition of the mixture determines the nature of the interaction between its components, which is confirmed by the observed precipitation during the formation of the M3 mixture. RBBR and brilliant green reacted strongly with each other, which was not observed in the case of mixtures with Congo Red. This phenomenon was not present during the preparation of mixtures M1 and M2.

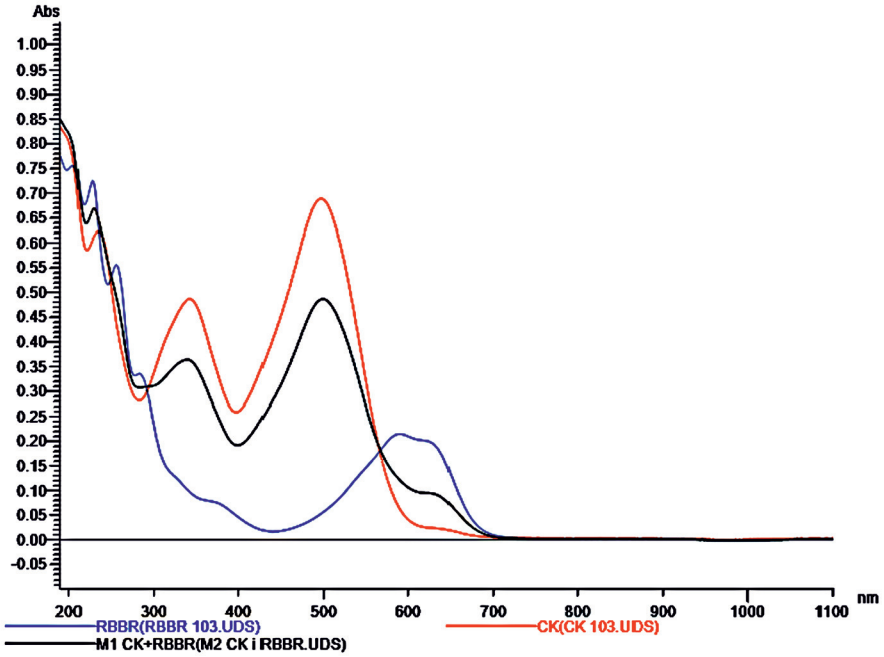


Fig. 1. UV-VIS spectra of a mixture of M1 and pure dye solutions

The first of the mixtures used in the study (M1) consisted of azo (CK) and anthraquinone (RBBR) dye. A characteristic feature of this mixture was the shift of λ_{max} from 490 nm characteristic of Congo red to 499 nm and the disappearance of the characteristic peak for RBBR at 593 nm (Fig. 1).

The M2 mixture was azo dye (Congo red) and triphenylmethane dye (brilliant green). Similarly to the M1 mixture, a shift of the λ_{max} peaks was observed from 490 nm characteristic for Congo red to 436 nm, and from 624 nm characteristic for brilliant green to 630 nm (Fig. 2). The λ_{max} peaks appearing on the graph are close to the peaks characteristic of brilliant green, which could suggest that the two dyes did not react well with each other, and the colour of the sample is dominated by triphenylmethane dye.

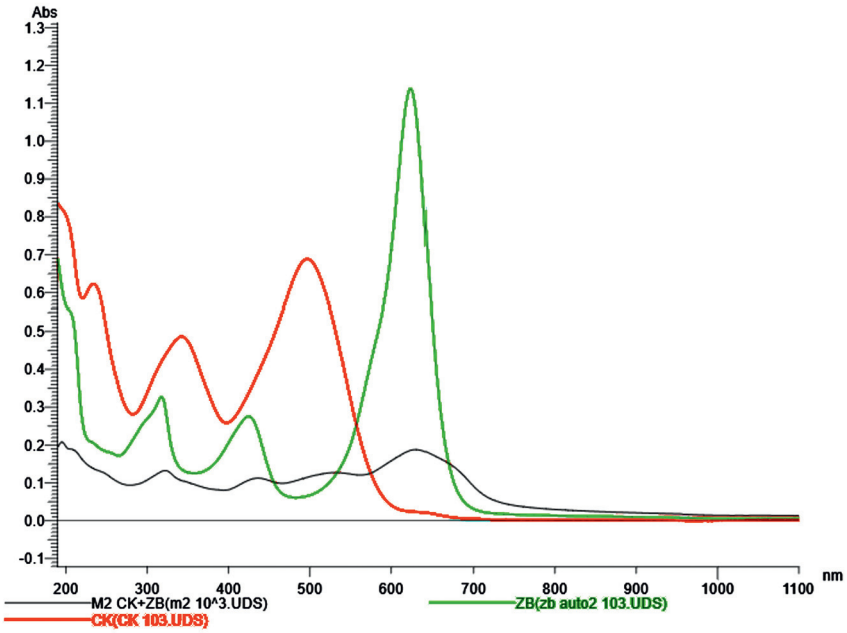


Fig. 2. UV-VIS spectra of a mixture of M2 and pure dye solutions

Interactions between dyes found in mixtures seem to confirm the ecotoxicity tests performed on *Daphnia magna* (Table 3). EC_{50} values for Congo red (12 mg/dm^3) and RBBR (7 mg/dm^3) allowed to classify these compounds as harmful and toxic respectively. However, for the two-component mixture of both dyes, the value EC_{50} – 5 mg/dm^3 was obtained, which is lower than for single dyes. This confirms that mixtures can be more harmful to the environment than substances that occur individually. Much worse toxicity results were obtained for the M2 mixture. Mixing toxic brilliant green with slightly less toxic Congo red resulted in an extremely toxic mixture. At a concentration of 1 mg/dm^3 M2 mixture, complete immobilization of test organisms was observed. Extreme toxicity of water samples with medium containing dye mixture has also been reported in previous studies on a mixture of azo dye Evans Blue and triphenylmethane dye Brilliant Green [20].

Table 3

Zootoxicity of dyes nad their mixture

Dyes/Mixtures	EC_{50} [mg/dm^3]	Class of toxicity
Congo Red	12	Hramful to quatic organisms
RBBR	7	Toxic
Brilliant Green	1.6	Toxic
Mixture 1	5	Toxic
Mixture 2	*	Very toxic

* Complete immobilization of tested organisms in concentration 0.001.

The high toxicity of dye mixtures may adversely affect the effectiveness of the treatment process of wastewater containing them.

Regardless of the concentration of the mixture, no colour removal was observed by the BWPH strain (at 228 nm) (Fig. 3a). Maximal removal (7 %) was observed at a concentration of 0.1 g/dm^3 and it was within the limits of measurement error. The lack of removal of the mixture at this wavelength by the mycelium of this strain confirms the difficulties in removing the mixtures by the fungi. At 340 nm and 499 nm (Fig. 3 b and c), it was observed that as the dye concentration increased, the dye removal by this strain increased. For a concentration of 0.1 and 0.12 g/dm^3 the removal of the mixture was 9 % at a wavelength of 340 nm, and for a wavelength of 499 nm ~19 %. At a concentration of 0.15 g/dm^3 it was 27 and 47 % respectively. The results obtained for the wavelength of 228 nm and negative removal values for the wavelengths of 340 and 499 nm at concentrations of 0.01–0.09 g/dm^3 suggest that there may be a chemical reaction of the mixture with metabolites produced by mycelium. This phenomenon has already been observed before [20]. The increase in colour for a concentration of 0.01 g/dm^3 M1 in more than 90 % regardless of the wavelength clearly indicates the interaction occurring in the samples and the appearance of new chemical bonds. Similar results were obtained for a second representative of the oyster mushroom (strain K4). No increase in colour was observed in the entire range of concentrations tested for the wavelength of 228 nm, and the highest degree of removal of 14 % was obtained at a concentration of 0.09 g/dm^3. A further increase in dye concentration resulted in a decrease in dye removal. For the remaining wavelengths and this strain the results were different. The percentage removal of M1 was from 12 to 21 % for a wavelength of 340 nm, with no correlation between the increase in dye concentration and colour removal. For the 499 nm wavelength in the concentration range of 0.01 to 0.09 g/dm^3, a decrease in the degree of decolorization was observed with increasing concentration (from 54 % to 19 %). For M1 concentrations of 0.12–0.15 g/dm^3, the removal was 40–46%. As the results of the study show, the efficiency of decolorization by both oyster mushroom strains is significantly different. This confirms earlier observations [22, 23], which showed that each strain has a different process character and ability to remove colour.

Completely different results of removal of the M1 mixture were obtained for the strain RWP17. As the concentration of the mixture increased, a decrease in colour removal was observed. For 228 nm wavelength from 76 % (0.01 g/dm^3) to just 8 % (0.15 g/dm^3), at 340 nm from 100 to 54 %, and for 499 nm from 95 % to 75 %. Especially for 340 nm and 499 nm waves, increasing concentration from 0.01 to 0.03 g/dm^3 drastically reduces the degree of removal. Further increasing the concentration of the mixture no longer resulted in drastic changes in the efficiency of decolorization. Such results suggest that for this strain the colour removal mechanism may be biochemical. This is indicated by a slightly decreasing degree of colour removal despite increasing dye concentrations. The results obtained at 228 nm suggest, however, that the process may also be of a sorption nature and result from the depletion of active sites in biomass. At this wavelength it was observed that as the concentration of the mixture increases, the degree of decolorization decreases. In the range of 0.03–0.15 g/dm^3, the linear nature of the decrease in colour removal along with the increase in the concentration of dyes was noted (R^2 = 0.9625).

In the case of the M2 dye mixture, as the concentration of the mixture increases, the degree of colour removal decreases regardless of the strain used for the study (Fig. 4), which is a reverse tendency than in the case of mixture M1. The BWPH strain better removed the mixture at concentrations from 0.01 to 0.09 g/dm^3 than the K4 strain belonging to the same species. Within this concentration range, the BWPH strain actually completely removed colour (322 nm wavelength). Strain K4 removed concentrations of M2 from 0.06 to 0.09 g/dm^3 much worse (<90%). Further increase in the concentration of dyes caused inhibition of the decolorization process by the BWPH mycelium. At a concentration of 0.15 g/dm^3 a colour increase in samples was obtained.

In samples with the K4 strain at 322 nm, the removal of the mixture at a concentration of 0.1 g/dm^3 was 23% higher than for the BWPH strain, and for 0.12 g/dm^3 it was almost 50 % higher. At the same time, the biomass concentration of the K4 strain is much lower than the biomass concentration of the BWPH strain. High removal efficiency at lower biomass concentration indicates that in the case of the K4 strain the process is biochemical, while in the case of the BWPH strain the process is based more on dye sorption. Even at a concentration of 0.15 g/dm^3, a positive colour removal result was obtained (11 % at 322 nm).

At 630 nm, however, the results of the colour reduction obtained for K4 strain were significantly lower than for the BWPH strain except concentration 0.15 g/dm^3. The observed decrease in process efficiency and an increase in the colour of the solution in case of strain BWPH may be associated with the saturation of active binding sites in biomass and the release of substances produced by the fungus into the solution.

A similar decrease in decolorization efficiency with increasing M2 concentration was observed for strain RWP17. This strain definitely the least removed the analyzed mixture, because for 322 and 436 nm wavelength, full decolorization was obtained only at 0.01 g/dm^3, and at 630 nm wavelength removal was only 76 %. Analyzing the results at 630 nm, decolorization was inhibited already at a concentration of 0.09 g/dm^3. At a concentration of 0.15 g/dm^3, no M2 mixture removal was observed.

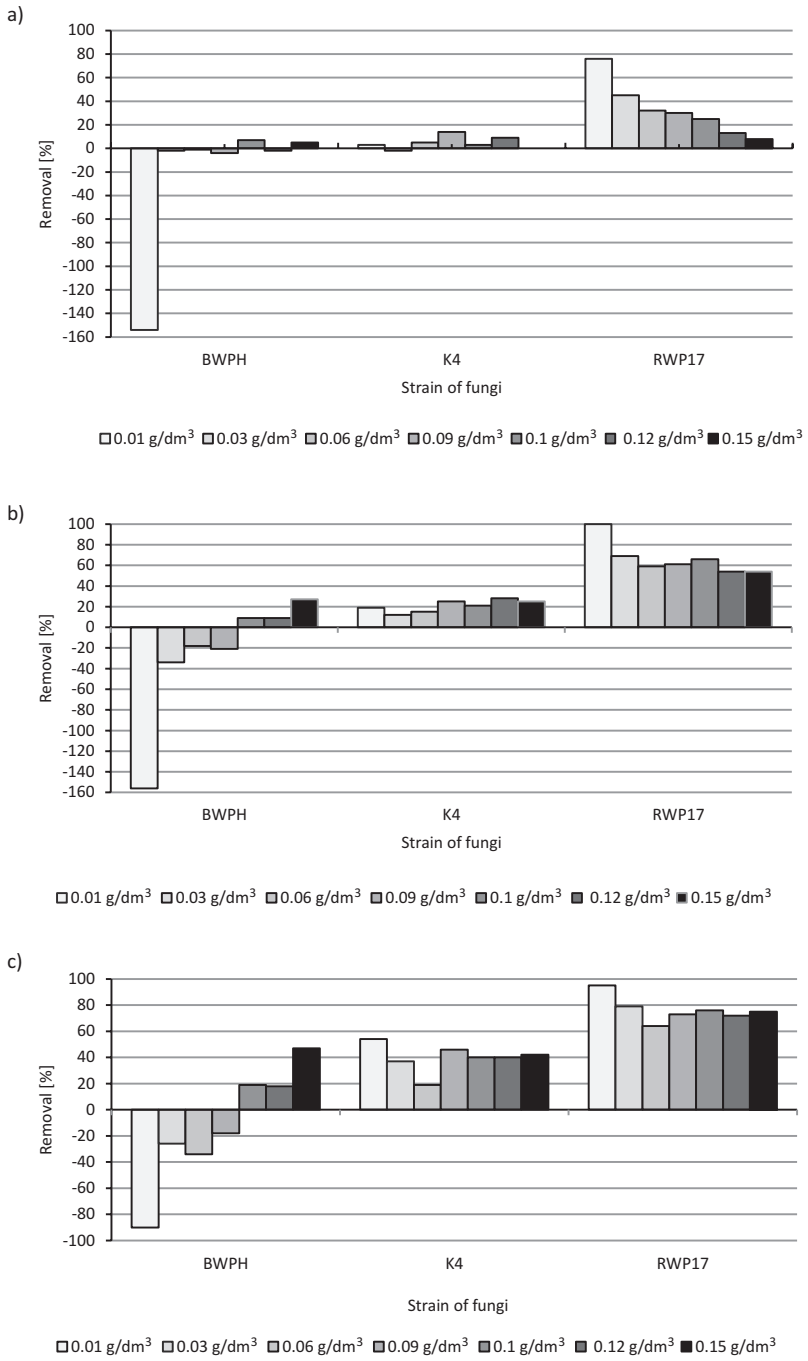


Fig. 3. Percentage removal of the M1 mixture at different wavelengths in samples with different strains of fungi (A – 228 nm, B – 340 nm, C – 499 nm)

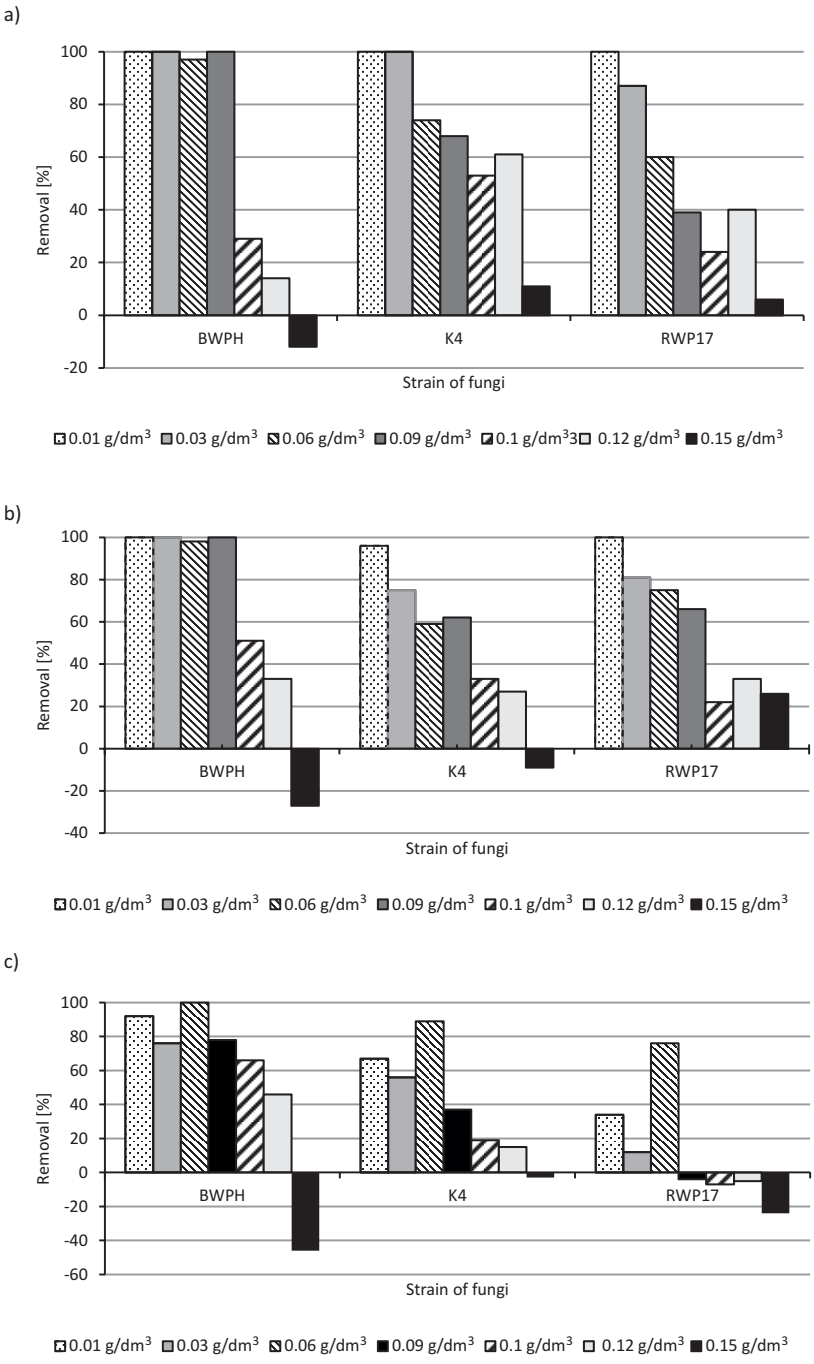


Fig. 4. Percentage removal of the M1 mixture at different wavelengths in samples with different strains of fungi a) 322 nm, b) 436 nm, c) 630 nm

Table 4

Concentration of dry biomass in samples [mg]

Mixture	Concentration of mixture in sample [g/dm^3]	BWPH	K4	RWP7
—	**0**	15.03	3.40	11.70
M1	**0.01**	27.43	4.63	14.00
	0.03	20.00	5.98	5.60
	0.06	22.40	11.65	8.80
	0.09	21.85	12.78	8.68
	0.10	18.53	11.55	8.28
	0.12	23.68	8.83	9.73
	0.15	20.80	9.33	5.70
M2	**0.01**	15.35	3.90	5.92
	0.03	9.30	8.10	8.31
	0.06	18.30	10.90	11.50
	0.09	17.90	12.60	15.89
	0.10	18.63	13.44	14.10
	0.12	17.10	14.91	15.53
	0.15	19.60	15.70	16.07

However, it should be noted that the efficiency of dye removal may also depend on the concentration of dry mycelium biomass. On the other hand, the presence of dyes in the medium may affect the intensity of mycelial growth in the samples. Both in the case of the M1 mixture and the M2 mixture it was found that they are not toxic to mycelium, and the efficiency of removing mixtures for any of the strains is not positively correlated with the biomass concentration (Table 4). The highest concentrations of mycelium were observed in samples with the BWPH strain, but in the case of the M1 mixture this strain was not able to remove the dyes contained in it regardless of the concentration. The dry matter concentration of the mycelium of the K4 and RWP17 strains was often more than half lower, but the dye removal, especially by the RWP17 strain, was at a high level. This suggests the involvement of biochemical dye transformation processes by both fungal strains. In the case of the RWP17 strain, it can be assumed that the process is mainly sorption, as indicated by the results discussed above (228 nm wavelength). However, the lack of a clear correlation between colour reduction and mycelium concentration in the samples confirms the simultaneous biochemical transformation during the process. For the M2 mixture (Table 4) the highest dry matter concentration was also observed for the BWPH strain. High biomass concentration was associated with high efficiency of decolorization for this mixture. The observed phenomenon of increasing the intensity of mycelium growth together with the increase in the concentration of the M2 mixture suggests that this mixture is not toxic to mycelium, and biotransformation and the sorption process took place in the samples. Biogens released into the substrate during dye biotransformation stimulated the biomass growth of this strain. A similar relationship was also observed for the

samples of the other two strains (K4 and RWP17). As the concentration of the mixture in the sample increased, the amount of dry matter increased, however, the degree of decolorization decreased.

Congo Red has been examined many times and it has been proved that it is a dye that is easily removed by fungi [23,24] *Aspergillus niger* mold fungus completely removed this dye from solutions in just 6 days [23, 24]. On the other hand, *Ceriporia lacerata* carried out the decolorization process with efficiency of over 90 % in just 48 hours [25]. Other dyes, such as RBBR and brilliant green were also examined and removed with varying efficacy by fungi [22, 26, 27]. For example, the strain MW84 classified as oyster mushrooms removed brilliant green in over 60% regardless of the composition of the medium used for growth. Another member of the same species decolorized the solutions only in the presence of high concentrations of organic compounds in the culture medium. Cassieri et al. [26] also obtained a large removal of RBBR. In the case of Bibi and Bhatti [27], the RBBR dye proved to be the best-removed reactive dye. Therefore, as shown by the results of studies of other authors, dyes selected to form mixture, despite toxicity and poor biodegradability should be effectively removed by fungi. Considering the above and the results of biodegradation tests presented for the M1 mixture, it should be stated that the reason for the difficulties in removing the mixtures is most likely the interaction between the dyes.

A decrease in the efficiency of decolorization in the case of dye mixtures in relation to pure substances has already been observed before [20]. The research was carried out on a two-component mixture of brilliant green and azo Evans blue using mycelium of strains BWPH and RWP17. The results obtained for such a mixture were consistent with the results for the M2 mixture, because it was observed that as the concentration of the mixture increased, the colour removal efficiency decreases. At the same time, it was observed that even a mixture of dyes 0.12 **g/dm^3** is removed by both strains in over 60 %, which is significantly different from the results obtained for the M2 mixture.

Summary and conclusion

The research confirms that the formation of mixtures of dyes from various classes, as well as their effect on living organisms requires intensive research. It was confirmed to both mixtures that they may be more toxic than individual dyes This is due to the changes occurring during the mixing of dyes, and confirmation was obtained especially on the example of the BWPH strain and the M1 mixture. It was observed that both mixtures by each of the strains used in the research were removed with different intensity, and the effectiveness of the process did not depend on the biomass concentration. The mechanism of mixtures removal is difficult to determine because of the complexity of the processes taking place in the samples. Significant differences in the intensity of the decolorization process confirm the need for further research on mixtures of dyes from various classes, including mixtures consisting of a greater number of dyes both in terms of environmental impact and potential options for their removal. It was also confirmed that strains of the same species differ significantly in both process efficiency and colour removal mechanism. Despite the not very high

colour removal of the M2 mixture, the RWP17 strain seems to be very promising for use in processes focused on removal of mixtures.

Acknowledgement

This research was supported by the 08/080/BK_19/0067 (BK-258/RIE8/2019) project

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MIESZANINY BARWNIKÓW – BADANIA ZOOTOKSYCZNOŚCI I USUWANIA PRZEZ WYBRANE SZCZEPY GRZYBÓW

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Abstrakt: Barwniki syntetyczne zaliczane są do zanieczyszczeń trudno usuwalnych ze środowiska, co wiąże się z ich toksycznym wpływem i niską podatnością na biodegradację. Jeszcze większy problem mogą stwarzać ich mieszaniny. Celem badań było zatem określenie toksycznego wpływu mieszanin (barwników z różnych klas) na organizmy wodne, a także efektywności usuwania ich przez grzyby zaliczane do podstawczaków. Badano dwuskładnikowe mieszaniny zawierały antrachinonowy remazolowy błękit brylantowy R (RBBR), azową czerwień Kongo i trófenylometanową zieleń brylantową (zakres stężeń 0.01–0.15 g/dm^3). Zootoksyczność określono z wykorzystaniem *Daphnia magna*. W badaniach wykorzystano trzy szczepy grzybów: BWPH i K4 zaliczane do gatunku *Pleurotus ostreatus* i RWP17 zaliczany do *Polyporus picipes*. Usunięcie barwników analizowano dla długości fal odpowiadającej najwyższej absorbancji mieszaniny.

Stwierdzono, że mieszaniny barwników są o wiele bardziej toksyczne dla organizmów żywych aniżeli pojedyncze substancje wchodzące w ich skład. W przypadku mieszaniny 1 (CK + RBBR) podczas testów dekoloryzacji obserwowano wzrost efektywność dekoloryzacji przez szczepy BWPH i K4 wraz ze wzrostem jej stężenia. Ta mieszanina była również dobrze usuwana przez szczep RWP17, a efektywność procesu dekoloryzacji malała wraz ze wzrostem stężenia (od 95 % do 75 %). Usuwanie mieszaniny 2 (CK + ZB) było bardzo duże dla szczepów zaliczanych do rodzaju *Pleurotus* – wzrost stężenia barwników powodował spadek efektywności procesu (z 100 % do 0 %).

Badania wykazały, że obecność mieszanin barwników w wodzie jest niebezpieczna dla organizmów w niej bytujących, a ich usuwanie stanowi duże wyzwanie. Efektywność procesu dekoloryzacji zależy od składu mieszaniny, jej stężenia, a także od wykorzystywanego w procesie szczepu.

Słowa kluczowe: mieszanina barwników, zootoksyczność, grzyby, dekoloryzacja, barwniki azowe, barwniki trójfenylometanowe, barwniki antrachinonowe