Lignicolous macromycetes: Potential Candidates for Bioremediation of Synthetic Dyes

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A simple and inexpensive method for biodegradation of synthetic dyes using new spontaneous macromycetes was elaborated. In the framework of this study, 54 lignicolous macromycetes (51 species isolated from natural habitats) were tested concerning the remediation potential against four different structural classes of synthetic dyes (azo, arylmethane, thiazine and anthraquinone). The screening was carried out both in solid (54 strains) and liquid (18 strains) media. The quantification degree of decolourisation was achieved by measuring the decolourisation halo diameter, and the effect of the dye on the fungal development has been established by measuring the fungal growth on supplemented media. The most effective species in bioremediation proved to be the common species of Bjerkandera adusta, Irpex lacteus, Daedaleopsis tricolor, Ganoderma resinaceum, Peniophora incarnata, Polyporus arcularius. To our knowledge, some of these species were studied for the first time regarding the biodegradation of these dyes. The same species that efficiently degraded the dyes on solid media also degraded the dyes at a high rate on liquid media. However, the remediation potential of the tested strains varied with the fungal species and dyes types. The Malachite green dye proved to be mycotoxic, while the Methyl blue was the most degraded dye.

Keywords: mycoremediation, synthetic dyes, biodegradation, lignicolous fungi

The synthetic dyes are chemical compounds with different structure that represent an important class of xenobiotic compounds widely used in various industrial activities, especially in the textile industry. The annual production of dyes is estimated at more than 10^6 tones worldwide, with over 100.000 chemically distinct dyes currently marketed [1]. A significant amount of synthetic dyes remain in the wastewater resulted from the technological processes of dyeing [2], which now becomes a major source of pollution of aquatic environment.

Highly chemically stable, the synthetic dyes are resistant to microbial degradation [3]. Various strategies for xenobiotics inactivation or degradation by physico-chemical methods have been elaborated but they are costly [4, 5] and sometimes ineffective [6]. Some researchers [7] used catalytic ozonation processes, but the operation costs were very high. The coupled chemical and biological treatment of xenobiotics was also tested [8]. The adsorption on different materials was also evaluated, though an appropriate sorbent is needed in this case and an additional treatment of the sorbent is necessary after usage [9].

The possibility of using a biological sorbent in order to recover the dyes with an appropriate sorbent was tested [10]. Due to the very large total surface of the mycelium, and therefore a wide contact zone, filamentous fungi have shown the best dye remediation ability among all the groups of organisms tested in biosorption processes [11].

The main goal is to eliminate the pollutants through biodegradation, lignicolous macromycetes demonstrating the highest degradative abilities as a result of their versatile and very complex enzymatic equipment, which allows them to degrade a wide range of remediation-reluctant organic compounds [12]. The enzymes commonly associated with such degradation processes involve laccases [13], manganese-dependent peroxidases, manganese-independent peroxidases, aryl alcohol oxidases [14], versatile peroxidases [15], cytochrom P450 monooxygenase etc. [16].

There are numerous studies on remediation and a special attention was paid to the isolates from common species (known as white rot fungi) from Polyporaceae [17, 18] or Meruliaceae [19]. Tests on species of commercial importance have also been performed although only a few studies dealt with species from other taxonomic families, such as Strophariaceae, Ganodermataceae, Fomitopsidaceae, Stereaceae etc. Nevertheless, many of these species can exhibit astonishing abilities to degrade xenobiotic compounds.

The emphasis of this work was to elaborate a straightforward and inexpensive method for the synthetic dyes biodegradation using new / less studied (*Daedaleopsis tricolor*, *Ganoderma resinaceum*, *Peniophora incarnata*, *Polyporus arcularius*, *Trametes gibbosa* and *Trametes suaveolens*) and common well-known macromycetes. We used spontaneous macromycetes species which may be easily obtained in order to reduce the costs of bioremediation. The relevant results obtained in the field of the synthetic dyes mycoremediation demonstrate the efficiency and cost effectiveness of using such methods to remove these pollutants.

Experimental part

Materials and methods

Fungal strains and inocula

The tested fungi were isolated using fruit bodies collected from dead wood found in forest habitats. The isolation process occurred under sterile conditions using the context mycelium of sporoms. The identification of the selected species was performed using classical macroscopical and microscopical methods [20-26], and the lyophilized specimens (UniEquip lyophilizator, UNICRYO

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MIC 4 L model, Planegg, Germany) were deposited in the Faculty of Biology Herbarium [I], Alexandru Ioan Cuza University of Iasi, Romania. All the tested strains and the corresponding herbarium number are listed in table 1. The nomenclature used in this paper is according to the Species Fungorum database [27].

Chemical reagents

Four different classes of dyes have been used: Congo red (CR) – azo, Methyl blue (MB), Malachite green (MG) and Crystal violet (CV) – arylmethane, Toluidine blue (TB) – thiazine and 1,2,4–trihydroxyantraquinone (TA) – anthraquinone. Stock solutions have been prepared using ethanol 96% for the TA and distilled water for the remaining dyes. All the reagents are of analytical grade and have been acquired from Merck KgaA, Germany.

Solid state culture

The adjusted method described by Levin et al. [28] has been used to perform the screening on solid media. Consequently, the 9 cm diameter Petri dishes filled with 20 mL MEG (malt extract - 10 g, dextrose – 5 g, agar – 15 g, distilled water – 1000 mL) have been employed for the purpose. The media have been supplemented with MB, CR, TB and TA in a final concentration of 100 ppm. Plates filled with non-supplemented media, inoculated with mycelium and incubated in similar conditions as the probe samples were used as control. All the media have been sterilized by autoclaving at 120°C in a 75 liters upright model autoclave (Raypas, Barcelona, Spain). Three replicates have been made for all the samples. Square fragments of agar plugs (1 cm^2) covered with mycelium, cropped from the periphery of the active growth state colonies have been used as inocula. All the samples have been incubated in the dark at 25°C, for 14 days.

Liquid state culture

The most efficient 18 isolates for the dye degradation have been also tested in liquid media. Organic media, containing malt extract (malt extract - 15 g/L, dextrose – 10 g/L) supplemented with MG, VC and TB at a final dye concentration of 100 ppm, have been distributed in test tubes, 10 mL each. The inoculation was performed with agar plugs of 2 cm^2, as presented above. Media have been sterilised at 120 °C and the samples incubated in the dark, at 25°C, for 10 days in an orbital shaker, at 180 rpm (GFL 3500, Germany). Three replicates were performed for each sample. The samples that presented highly different values of decolourisation were considered errors and redone.

Analytical methods and measurements

On solid media, the fungal tolerance to the toxicity of dyes have been evaluated based on measuring the growth of the fungi on the supplemented media (the diameters of the colonies developed in the unit of time) compared to the control.

The diameter of the decolourisation halo formed around the colonies was used for the estimation of the dye remediation level. The cultures have been analysed after 7, 10 and 14 days of incubation, the diameters of the halos and colonies being measured. All the measurements of the colonies and halos started at the value 1.00, considered the surface covered by the inoculum. The final results represent the average of the three replicates, with a standard error less than 5%. The isolates have been classified based on their decolourisation efficiency, in 5 classes: class a – 10-20 mm diameter; class b – 21-35 mm diameter; class c – 36-55 mm diameter; class d – 56-75 mm diameter and class e – 76-90 mm diameter. A number of points have been assigned for each class, 1 point for class a, 5 points for class e, and the sum of the points for any dye gave the total score of an isolate.

For the evaluation of the dyes degradation rate, a ratio between the halo diameter and the fungal colonies diameter has been calculated, ratio noted forward with *R* (table 1). Only the measurements performed after 10 days of incubation have been considered for calculation due to complete decolourisation of the plates after this time by several isolates.

Spectrophotometric studies

The liquid media was centrifuged at 5000 rpm (MICRO 22 R, Hettich, Minnesota, USA) after 10 days of incubation and the resulted supernatant was photometrically evaluated using an UV-Vis spectrophotometer (Pharmaspec UV-1700 model, Shimadzu Europe Ltd., Manchester, UK). The absorbance was measured at the wavelength corresponding to the maximum absorption for each dye. The decolourisation rate was calculated according to the equation (1).

$$\%\ discoloration = \frac{(A-B)}{A} * 100 \qquad (1),$$

where A is the absorbance at λ_{max} of the control supernantant and B is the absorbance at λ_{max} of the samples supernatant [29]. All the calculations were verified using the calibration curve. An electronic *p*H/ion-meter (model INOLAB, WTW, Weilheim, Germany) was used to measure the *p*H before and after incubation time. The average difference between the *p*H values of the treated samples and the controls was calculated.

Statistical analysis

A Principal Component Analysis (PCA) was run to evaluate the susceptibility of dyes to degradation, using the covariance as the similarity index. The values used in the test were the measured halos diameters for the four tested dyes (for all the strains). The Kruskal-Wallis test was run as an additional method for comparison of k samples. The Kruskal-Wallis test allowed us to evaluate two properties related to the fungi-dyes interaction: the ability of fungi to degrade dyes [by comparing halo degradation diameters with two values used as referee: 1.00 (minimum – the absence of the degradation) and 9.00 (maximum – complete degradation)] and the fungal tolerance degree to toxic effect of dyes (using as variables the diameters of fungal colonies grown on supplemented and non-supplemented media). The statistical analysis was performed using the XLSTAT 2012 software (trial version).

Results and discussions

The screening on solid media showed significant differences among the isolates concerning their dye degradation efficiency (Table 1). The common species included in the Meruliaceae family (*Bjerkandera adusta* 1 and 2, *Irpex lacteus* 1 and 2) were proved to be the most efficient, these results being in accordance with those reported by other authors in related cases [30]. We also noticed that species from the Polyporaceae family (*Coriolopsis gallica*, *Lenzites betulina*, *Trametes gibbosa*, *T. hirsuta* and *T. Pubescens*), exhibited high degradative abilities. A reasonable explanation for the high efficiency of *Bjerkandera*, *Irpex* and *Trametes* species in dye degradation, could be associated with their versatile enzymatic system involved in the xenobiotics degradation [13, 15, 31].

ISOLATE	SPECIMEN*	TB**	TA**	CR**	MB**	SCORE***
***Bjerkandera adusta* 1**	**[I 137350]**	1.00 **e**	0.96 **e**	0.96 **e**	1.00 **e**	**20.00**
***Irpex lacteus* 2**	**[I 137351]**	0.94 **e**	1.00 **e**	0.97 **e**	1.00 **e**	**20.00**
***Bjerkandera adusta* 2**	**[I 137352]**	1.00 **e**	0.89 **e**	0.83 **d**	1.00 **e**	**19.00**
***Irpex lacteus* 1**	**[I 137353]**	0.90 **e**	090 **d**	0.98 **e**	1.00 **e**	**19.00**
Trametes gibbosa	**[I 137354]**	1.00 **e**	0.85 **d**	0.89 **e**	1.00 **e**	**19.00**
Lenzites betulina	**[I 137355]**	0.97 **e**	0.79 **c**	0.86 **e**	1.00 **e**	**18.00**
Trametes hirsuta	**[I 137356]**	1.00 **e**	0.87 **d**	0.91 **d**	1.00 **e**	**18.00**
Trametes pubescens	**[I 137357]**	0.50 **c**	1.00 **e**	0.92 **e**	1.00 **e**	**18.00**
Coriolopsis gallica	**[I 137358]**	0.28 **b**	0.89 **e**	0.90 **e**	1.00 **e**	**17.00**
Trametes suaveolens	**[I 137359]**	0.47 **c**	0.71 **d**	0.91 **e**	1.00 **e**	**17.00**
***Trametes versicolor* 2**	**[I 137360]**	0.76 **d**	0.56 **c**	0.86 **e**	1.00 **e**	**17.00**
Peniophora incarnata	**[I 137361]**	0.70 **d**	1.04 **d**	0.75 **c**	1.00 **e**	**16.00**
Polyporus arcularius	**[I 137362]**	1.00 **e**	1.00 **c**	0.77 **c**	1.00 **e**	**16.00**
***Trametes versicolor* 1**	**[I 137363]**	0.77 **d**	0.53 **c**	0.65 **d**	1.00 **e**	**16.00**
Bjerkandera fumosa	**[I 137364]**	0.67 **d**	1.00 **c**	0.84 **c**	0.75 **d**	**14.00**
Daedaleopsis tricolor	**[I 137365]**	0.38 **b**	0.83 **c**	0.82 **c**	0.82 **e**	**13.00**
Ganoderma adspersum	**[I 137366]**	0.11 **a**	1.00 **d**	0.90 **c**	0.95 **e**	**13.00**
Ganoderma resinaceum	**[I 137367]**	0.11 **a**	0.83 **d**	0.64 **c**	1.00 **e**	**13.00**
Phellinus conchatus	**[I 137368]**	**0.87 c**	**1.05 c**	**0.95 c**	**0.97 d**	**13.00**
Cyathus striatus	[I 137369]	0.17 **a**	0.93 **c**	0.64 **c**	0.88 **d**	11.00
Gymnopilus junonius	[I 137370]	0.20 **a**	1.00 **c**	0.57 **c**	0.81 **d**	11.00
Hypholoma fasciculare	[I 137371]	0.16 **a**	1.00 **c**	0.71 **c**	1.00 **d**	11.00
Hypholoma lateritium	[I 137372]	0.16 **a**	1.00 **c**	0.95 **c**	1.00 **d**	11.00
Phellinus pomaceus	[I 137373]	0.24 **a**	1.00 **c**	0.85 **c**	0.99 **d**	11.00
Pleurotus ostreatus	[I 137374]	0.49 **b**	1.00 **c**	021 **a**	0.98 **e**	11.00
Peniophora quercina	[I 137375]	0.16 **a**	0.59 **c**	061 **b**	0.80 **d**	10.00
Fomes fomentarius	[I 137376]	0.17 **a**	1.00 **b**	083 **c**	0.88 **c**	9.00
Xylaria polymorpha	[I 137377]	0.16 **a**	0.79 **c**	020 **a**	1.08 **d**	9.00
Flammulina velutipes	[I 137378]	0.18 **a**	0.20 **a**	021 **a**	1.00 **e**	8.00
Ganoderma applanatum	[I 137379]	0.26 **a**	1.00 **c**	037 **a**	1.00 **c**	8.00
Ganoderma lucidum	[I 137380]	0.13 **a**	0.26 **a**	064 **c**	0.62 **c**	8.00
Phellinus igniarius	[I 137381]	0.38 **a**	0.99 **b**	048 **a**	0.93 **d**	8.00
Schizophyllum commune	[I 137382]	0.11 **a**	0.13 **a**	012 **a**	1.00 **e**	8.00
Inonotus hispidus	[I 137383]	0.30 **a**	1.04 **b**	040 **a**	1.09 **c**	7.00
Lycoperdon pyriforme	[I 137384]	0.31 **a**	1.26 **b**	0.61 **a**	1.04 **c**	7.00
Royoporus badius	[I 137385]	0.26 **a**	0.83 **a**	0.88 **b**	1.08 **c**	7.00
Skeletocutis alutacea	[I 137386]	0.11 **a**	0.22 **a**	0.16 **a**	0.74 **d**	7.00
Armillaria mellea	[I 137387]	0.67 **a**	1.53 **b**	0.67 **a**	1.00 **b**	6.00
Auricularia auricula-judae	[I 137388]	0.71 **a**	1.39 **b**	1.00 **a**	0.82 **b**	6.00
Auricularia mesenterica	[I 137389]	0.44 **a**	1.06 **b**	0.50 **a**	0.96 **b**	6.00
Kuehneromyces mutabilis	[I 137390]	0.16 **a**	0.30 **a**	0.25 **a**	0.51 **c**	6.00
Pholiota alnicola var. *salicicola*	[I 137391]	0.20 **a**	1.00 **b**	0.32 **a**	0.63 **b**	6.00
Pholiota aurivella	[I 137392]	0.27 **a**	0.36 **a**	0.32 **a**	0.74 **c**	6.00
Hemipholiota populnea	[I 137393]	0.83 **a**	1.00 **a**	1.00 **a**	1.08 **b**	5.00
Daedalea quercina	[I 137394]	0.13 **a**	0.17 **a**	0.13 **a**	0.14 **a**	4.00
Daedaleopsis confragosa	[I 137395]	0.13 **a**	0.13 **a**	0.22 **a**	0.11 **a**	4.00
Fomitopsis pinicola	[I 137396]	0.11 **a**	0.32 **a**	0.11 **a**	0.11 **a**	4.00
Inonotus cuticularis	[I 137397]	0.56 **a**	0.83 **a**	0.63 **a**	0.56 **a**	4.00
Merulis tremellosus	[I 137398]	0.34 **a**	0.63 **a**	0.27 **a**	0.24 **a**	4.00
Plicaturopsis crispa	[I 137399]	0.59 **a**	0.32 **a**	0.71 **a**	0.42 **a**	4.00
Postia caesia	[I 137400]	0.55 **a**	0.83 **a**	0.53 **a**	0.31 **a**	4.00
Postia stiptica	[I 137401]	0.78 **a**	0.83 **a**	0.56 **a**	0.48 **a**	4.00
Stereum hirsutum	[I 137402]	0.42 **a**	0.49 **a**	0.47 **a**	0.40 **a**	4.00
Xylobolus frustulatus	[I 137403]	0.38 **a**	0.47 **a**	0.26 **a**	0.30 **a**	4.00

Table 1
THE DEGRADATION OF THE SYNTHETIC DYES AFTER 10 DAYS OF INCUBATION

** the number of the specimen in the Herbarium*
*** the ratio between halo and fungal colony diameters and degradation efficiency classes – a: 10-20 mm diameter; b: 21-35 mm diameter; c: 36-55 mm diameter; d: 56-75 mm diameter; e: 76-90 mm diameter*
**** the efficiency score of the isolates*
***** in bold are marked the isolates that presented the highest values of the degradation processes*

In our studies special attention was paid to *Trametes suaveolens, Peniophora incarnata, Polyporus arcularius, Ganoderma adspersum and G. resinaceum* species, taking into consideration that the number of studies dedicated to the use of these species is very limited.

Unexpectedly, common species, such as *Fomes fomentarius* or *Pleurotus ostreatus*, presented moderate degradative rates; in similar cases [14, 32, 33] the authors found that *Pleurotus* were very active. We believe that these differences are related to the structure of the dye which is different (despite the fact that they belong to the same class). Additional data may be obtained after employing other analytical procedures (such as chromatography) in order to assess the structures of dye metabolites.

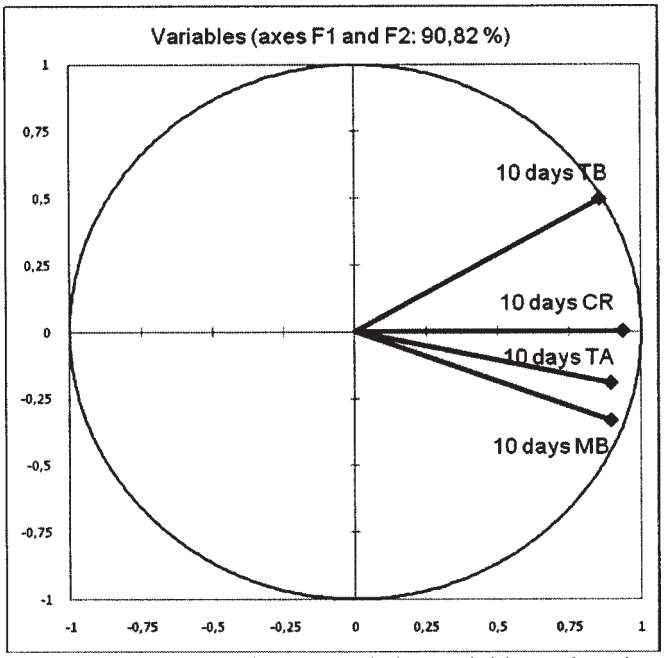

Fig. 1. The circle of correlation between the factors and the variables. The plane formed by the first two principal factors reproduces 90.82 % of the original information

High differences between the dye degradation rate and growth rate were observed even for the same isolate. However, species like *Armillaria mellea*, *Auricularia auricula-judae* and *Lycoperdon pyriforme* showed high rate of degradation of TA compared to the growth rate on media containing the dye (table 1).

Analysing the ratio between the degradation rate and the growth rate we can observe a general retardation of the degradative processes compared to the hyphal growth. This lag may be caused by the time needed for the accommodation to substrate, the induction of enzymatic system and the diffusion of the enzymes into the substrate (this point of view is also sustained by the low degradation rates registered after 7 days of incubation). The most efficient strains degraded the dye concomitantly with the hyphal growth. On TA supplemented media, several isolates degraded the dye before the hyphal growth. Taking into consideration these results with the proved inhibitory effect of TA, it may be concluded that these strains needed the elimination or conversion of the dye prior to the fungal development.

The Principal Component Analysis showed significant differences between the degradation rates of the four dyes (p-value < 0.0001). The first two factors generated almost 91% of the variability and the first factor alone generated approximately 80% of the results, which means that the representation is of high quality. At the same time, the KMO - Kaiser-Meyer-Olkin measure of sampling adequacy was 0.808. Analysing the correlation circle (fig. 1), we may observe that the variables are far from the centre, therefore the projection is secure. There is a positive correlation between CR, TA and MB. TB is not correlated with MB and TA and presents a weak correlation with CR. The particular position of TB on the correlation circle proves an atypical behaviour of the dye. This particular behaviour is also proved by the way the decolourisation occurred on several plates (irregular decolourisation). The involved mechanism is not yet known, but a possible explanation could be represented by the hyphal differentiation and the secretion of the enzyme responsible for the dye degradation only by few hyphae. This might be true, taking into consideration that many of the tested strains have a dimitic or trimitic hyphal system.

The positive correlation between TA and MB or CR demonstrates the existence of similar patterns, probably due to the synthesis of the same enzymes involved in the degradation of the dyes.

After running the Kruskal-Wallis test with the halos diameters values and the two opposite values of the ideal controls as variables in the test, remarkable differences between the susceptibility of the dyes to degradation were observed (p-value < 0.0001). This test takes into account more information than the position given by the mean; it also allows the ranking of the variables, therefore the ranking of the dyes based on their degradation rate.

The Multiple pairwise comparisons (table 2) revealed a separation of the data series (the variables) in four groups, two of them corresponding to the ideal controls and the other two corresponding to the dyes. There are significant differences between the dyes and the two controls. The susceptibility to degradation is in the following order: TB<CR<TA<MB.

The results registered for MB represent a separate group.The fast degradation of MB by the majority of the strains (table 2) can be correlated with the absence of the toxicity of this dye (table 3). Various strains degraded all the dyes (*Bjerkandera adusta* 1 and 2, *Irpex lacteus* 1 and 2, *Trametes gibbosa*) while other strains degraded fast only three of the tested dye (*Trametes pubescens*).

A comparison similarly to the previous was made using the diameters of the colonies grown on supplemented and non-supplemented media. This test revealed a strong inhibition of the TA and CR dyes over fungal development (table 3), a moderate inhibition displayed by TB and a slow inhibition exhibited by MB (p-value < 0.0001). The relative degradation resistance of CR and TA might be due to the mycotoxicity exhibited by these dyes. Different authors reported strong degradations of CR [2, 34]. The low degradation rate of TB comparing with MB, although this dye did not present a strong inhibitory effect, may be

Table 2
DEGRADABILITY OF THE DYES ASSESSED BY MULTIPLE PAIRWISE COMPARISONS (KRUSKAL-WALLIS TEST, XLSTAT SOFTWARE)

SAMPLE	SUM OF RANKS	MEAN OF RANKS	GROUPS
CONTROL 1	3807.000	70.500	A
10 days TB	6784.000	125.630	B
10 days CR	7597.500	140.694	B
10 days TA	8157.000	151.056	B
10 days MB	10833.500	200.620	C
CONTROL 2	15471.000	286.500	D

Table 3
TOXICITY OF THE DYES ASSESSED BY MULTIPLE PAIRWISE COMPARISONS (KRUSKAL-WALLIS TEST, XLSTAT SOFTWARE)

SAMPLE	SUM OF RANKS	MEAN OF RANKS	GROUPS
10 days TA	83.000	1.537	A
10 days CR	115.500	2.139	A
10 days TB	171.500	3.176	B
10 days MB	211.000	3.907	B, C
10 days CONTROL	229.000	4.241	C

ISOLATES	MG	TB	CV
Bjerkandera adusta 1	20.67	76.07	28.94
Bjerkandera adusta 2	23.56	81.21	19.64
Bjerkandera fumosa	21.96	46.70	26.12
Coriolopsis gallica	26.10	62.95	35.71
Daedaleopsis tricolor	15.71	34.45	28.73
Ganoderma adspersum	23.02	56.94	26.54
Ganoderma resinaceum	33.33	63.73	34.21
Irpex lacteus 1	24.36	54.88	33.83
Irpex lacteus 2	10.99	49.87	31.44
Lenzites betulina	17.09	43.82	54.66
Peniophora incarnata	10.19	65.34	27.91
Polyporus arcularius	80.58	36.62	28.19
Trametes gibbosa	21.71	67.33	25.35
Trametes hirsuta	32.74	41.21	47.52
Trametes pubescens	26.10	64.33	21.73
Trametes suaveolens	91.20	58.06	24.51
Trametes versicolor 1	27.44	56.43	34.59
Trametes versicolor 2	24.83	49.22	17.04

Table 4
THE DEGRADATION OF THE SYNTHETIC DYES ON LIQUID MEDIA (% DEGRADATION)

caused by the same mechanism presented above. However, additional studies are necessary in order to establish the optimal conditions for the synthetic dye mycoremediation and to understand all the mechanisms involved in these processes and also for the elucidation of the mechanism that gives the particular behaviour of the TB.

The results concerning the dyes degradation on shaken liquid media were very different for each of the isolates (table 4) for the three tested dyes. A possible explanation of this behaviour might be related to the structure of the dyes, as in similar cases [35].

TB was the most frequently degraded dye, with an average degradation rate of 55.50%, the most efficient isolates being *Bjerkandera adusta* 1 and 2, *Trametes gibbosa*, *T. pubescens* and *Peniophora incarnata*. In other words, the hierarchy of the effective strains remained unchanged for the TB. CV was degraded with an average degradation rate of 29.60%, the most efficient strains being *Lenzites betulina*, *Trametes hirsuta*, *Trametes versicolor* 1, *Irpex lacteus* 2, *Coriolopsis gallica* and *Ganoderma resinaceum*. MG was degraded at a low rate by the majority of the strains, the average rate being 26.27%. However, several isolates, such as *Trametes suaveolens* and *Polyporus arcularius* degraded the dye very fast. For CV the most efficient strains were also situated near the top in the previous classification. These results are in accordance with those reported in similar cases [3]. The most efficient strains for the MG degradations were others than the expected ones [28].This proves the necessity for testing as many isolates and fungal species as possible for the purpose of the xenobiotic bioremediation. Analysing the *p*H modifications during the fungal growth we can observe that these changes are not significant enough to influence the decolourisation processes.Taking into consideration the costs of the processes and the efficiency of the dye transformation, a liquid system should be used for bioremediation purposes.

By measuring the *p*H values before inoculation and after incubation we observed a slow acidification of the media due to fungal activity. The maximum calculated *p*H differences were 0.56 for MG, 0.72 for TB and 0.73 for CV, with average values of 0.24 for MG, 0.28 for TB and 0.51 for CV. In few cases a small increase of *p*H was observed (for MG).

Conclusions

To summarize, it can be established that these results highlighted the abilities of different macromycetes species to degrade synthetic dyes and, consequently, their potential in environment bioremediation. This bioremediation method is simple and inexpensive using spontaneous macromycetes species. The bioremediation ability varies with the isolates, species and dye types. The most effective species in bioremediation were proved to be the common species of *Bjerkandera adusta, Irpex lacteus*, *Trametes sp.*, *Daedaleopsis tricolor*, *Ganoderma resinaceum*, *Peniophora incarnata* and *Polyporus arcularius*. The same species that efficiently degrade the dyes on solid media also degrade the dye at a high rate on liquid media. Significant differences between the susceptibility to degradation were observed among the dyes, the most degraded being MB. The less degraded dye was TB for the solid media and MG for the liquid media. The dyes inhibited the fungal development in a different manner. The less toxic dye was MB, and the dyes that strongly inhibited the growth of the colonies were TA, CR and MG. TB presented an atypical behaviour, inhibiting slightly the fungal growth.

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