# Evaluation of decolorisation abilities of some textile dyes by fungal isolates

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**REZUMAT – ABSTRACT**

**Evaluarea abilităţilor de decolorare a unor coloranți textili cu izolate fungice**

*Nouă tulpini fungice izolate şi identificate anterior din efluenţi rezultaţi în urma etapelor de finisare textilă au fost investigate pentru abilitatea acestora de decolorare a trei coloranţi textili Bemacid, în soluţie apoasă. Izolatele fungice identificate aparţin următoarelor grupuri: Trichoderma parceramosum, Trichoderma reesei, Trichoderma longi, Polyporus squamosus şi Fusarium oxysporum, alături de Aspergillus niger (IMI 45551) folosită ca tulpină de referinţă, de colecţie. Maximele de absorbanţă în regiunea vizibilă, pentru fiecare colorant, au fost determinate spectrofotometric şi reducerea reziduală a concentraţiei coloranţilor a fost determinată la 500 nm pentru Bemacid ROT, 370 nm pentru Bemacid GELB şi 590 nm pentru Bemacid BLAU. Gradul de puritate al coloranţilor a fost determinat prin Cromatografie pe Strat Subţire (TLC), iar testele de decolorare au fost efectuate în substrat nutritiv, în cinci şarje simultane, la 3, 6, 9, 12 şi 15 zile, în triplicat, rezultatele fiind exprimate ca medie a valorilor triplicate pentru fiecare combinaţie de colorant şi tulpină. Analiza cantitativă a decolorării soluţiilor a fost efectuată prin spectrometrie în UV-VIS, cuantificându-se gradul de decolorare de-a lungul perioadei de incubare, la 29°C, concentraţia reziduală a fiecărui colorant variind de la 20,98% la 98,02% pentru Bemacid ROT, 43,5% la 96,06% pentru Bemacid GELB şi 35,68% la 98,38% pentru Bemacid BLAU, promovând astfel abordarea biologică de tratare a apelor uzate cu ajutorul fungilor filamentoşi ca soluţie eficientă, rentabilă şi prietenoasă mediului.*

*Cuvinte-cheie: decolorare, fungi, lacază, coloranţi textili*

**Evaluation of decolorisation abilities of natural fungal isolates**

*Nine previously isolated and identified fungal strains from post-finishing textile effluents were investigated for their decolorisation ability of three Bemacid textile dyes, in aqueous solution. Identified fungal isolates belong to the following groups: Trichoderma parceramosum, Trichoderma reesei, Trichoderma longi, Polyporus squamosus and Fusarium oxysporum, along with Aspergillus niger (IMI 45551), used as a reference collection strain. Maximum absorbance peaks in visible region, for each dye, were assessed spectrophotometrically and dye residual concentration reduction were assessed at 500nm for Bemacid ROT, 370nm Bemacid GELB and 590nm Bemacid BLAU. Purity screening of the dyes was assessed by Thin Layer Chromatography (TLC), and decolorisation assays were carried out in nutritive media, in 5 simultaneous batches, for 3, 5, 9, 12 and 15 days, with each batch run in triplicate, and results expressed as mean of triplicate values for each combination of strain and dye. Quantitative analysis of solutions decolorisation was carried out via UV-VIS spectrophotometry assessment, quantifying decolorisation degree over post-incubation period at 29°C, each dye residual concentration reduction ranging from 20.98% to 98.02% for Bemacid ROT, 43.5% to 96.06% for Bemacid GELB and 35.68% to 98.38% for Bemacid BLAU, thus promoting biological approach of wastewater treatment with the aid of filamentous fungi as an efficient, cost effective and environmental friendly solution.*

*Keywords: decolorisation, fungi, laccase, textile dyes*

## INTRODUCTION

Textile industry generates large volumes of wastewater effluents, colorization degree representing the main pollutant parameter of the effluents [1–3]. Textile industry wastewaters present a complex matrix, containing a mixture of dyes with other organic and inorganic compounds, treating processes becoming of great complexity due to residual substances that are very hard to remove by stages of classical mechanical-biological processing [4]. Discharge of untreated textile effluents into main water bodies can often lead to limitation of re-oxygenation capacity of the effluents, modification of sunlight absorption rates which affects photosynthetic activity of aquatic systems, thus leading to high toxicity [5]. Pollution degree of textile effluents increases significantly with the use of wide varieties of textile dyes, strongly influenced by chemical structure of their chromophore groups, with azo-dyes being the largest group of synthetic textile dyes that are widely used and released into the environment, thus leading to need of viable and economical efficient bioremediation techniques.

The presence of dyes in textile industry generated waste waters, even in low concentrations of 1 mg/L, leads to alteration of esthetic and transparence properties of public water effluents, with direct repercussions on the environment [6]. Synthetic dyes used in the textile industry cannot be easily biodegraded, due to their complex aromatic molecular structure, which

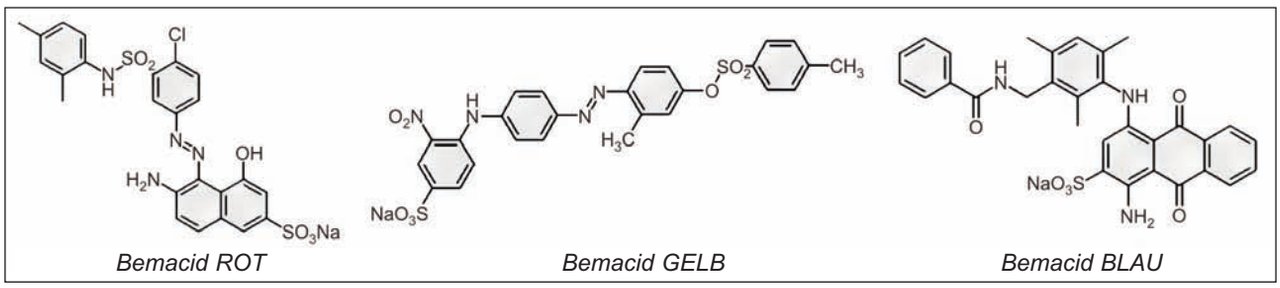


Fig. 1. Bemacid dyes structural formulas

furthermore renders them as resistant to conventional microbial treatment techniques [7]. Due to wide spread of microbial mediated treatment processes, decolorisation of azo-dyes textile effluents using fungi emerges as a new and efficient treatment method, due to their high mineralizing capacity and enzyme mediated oxidizing properties of certain pollutants [8, 9–12].

Microbial decolorisation of textile wastewater effluents is a cost effective and environmental friendly process, representing an efficient tool of controlling pollution generated by textile industry. Biological approach of certain pollutants removal from textile effluents is based on materials transfer from the water to the microbial cell, and the other way around, through interfacial contact or adsorption/desorption governed processes. The adsorbed compounds are involved in enzymatic reaction that takes place in multiple stages [13]. Bioaccumulation of pollutants takes place in metabolically active cells, and involves slow associated processes, with the implication of $H^+$-ATP-ase as a process mediator [14]. All transfer processes involve chemical interactions with certain functional groups, specific to outer layers of microbial cells: carboxylic groups (-COOH), amino groups ($-NH_2$), amide groups ($RCONR_2$), hydroxyl groups (-OH), sulfhydryl groups (-SH) and phosphate groups ($PO3^{4-}$) [15]. Fungal enzymes have an important role in increasing the activity rate of microorganisms in stages of bioremediation, and to mediate decomposing of organic matter by hydrolytic and oxidation-reduction processes, and further usage as nutritive substrates for microbial populations used in the treatment processes [16–17]. Enzymes act as biochemical catalysts, increasing the rate of chemical reactions that occur in all biological purification stages [18]. Biotechnological stages of microbial or enzymatic treatment of wastewaters are effective alternatives for treating effluents with high content of azo-dyes using a variety of microorganisms, such as bacteria, fungi, yeasts, actinomycetes and algae [19–20].

## EXPERIMENTAL WORK

### Materials and methods

#### *Fungal strains*

Nine fungal strains were used in the decolorisation experiments, most of them being isolated from a wastewater source resulted from textiles finishing processes and previously identified, by molecular methods, as following: I1, I2 and I3 belong to *T.parceramosum/T.reesei/T.longi group; I4 = Aspergillus niger IMI 45551* (ATCC 6275)*; I5 = Polyporus squamosus; I6 = Fusarium oxysporum; I7 and I8 =* yeast-like unidentified strains; I9 = *Trichoderma atroviride* [21]. Fungal strains were grown on Czapek-Dox broth from Fluka Analytical (30 g/L sucrose, 3 g/L sodium nitrate, 0.5 g/L magnesium sulfate, 0.5 g/L potassium chloride, 1.0 g/L potassium phosphate dibasic, 0.01 g/L ferrous sulfate, *p*H 7.3 at 25°C), incubated at 28°C for 14 days, before mixing with the dyes, and all samples run in triplicates.

#### *Textile dyes*

Three industrial azo-dyes were used in the decolorisation experiments, respectively Bemacid ROT N-TF(CAS EINECS: 276-115-7), $C_{24}H_{20}ClN_4NaO_6S_2$, M = 583.0 g/M (7), Bemacid GELB N-TF(CAS EINECS: 235-406-9), $C_{25}H_{19}N_4NaO_8S_2$, M = 590.56 g/M (8) and Bemacid BLAU N-TF(CAS EINECS: 267-224-0), $C_{31}H_{28}N_3NaO_6S$, M = 593.63 g/M (9), produced by BEZEMA AG Company, with structural formulas shown in figure 1.

Two stock solutions were prepared for the assays, for each dye, one stock solution for calibration curves, with concentration of 200 mg/L, and one stock solution for decolorisation assays, with concentration of 5 g/L. Both stock solutions were prepared by stirring the dye, at 500 rpm, in 1000 mL Czapek-Dox nutritive broth at 30°C for 3 hours, and then sterilized at 121°C for 15 minutes. Maximum absorbance of dyes was measured with Lambda 950 UV-VIS spectrophotometer and selected to plot the calibration curves used to quantitatively evaluate the decolorisation induced by microorganisms. In order to calculate the dye concentration with a greater accuracy, two calibration curves were carried out (data not shown), by splitting the concentration domain of 0–200 mg/L into two ranges for each dye, respectively from 0 to 50 mg/L, in six points, and from 50 to 200 mg/L, in four points, instead of raging the 0–200 mg/L interval in 4 standard points, thus obtaining correlation factors above 0.9892 for the lower range interval and above 0.9969 for the higher range interval.

## Methods and analysis

### *Decolorisation studies*

For decolorisation studies, microbial strains were grown for 14 days at 28°C and allowed to reach metabolic maturity. Stock dye solution, of 5 g/L, concentration, was prepared in nutritive broth, and sterilized at 121°C for 15', and afterwards 2 mL of stock solution was pippeted into the Erlenmeyer flasks, with strains, for a final volume of 50 mL and final dye concentration of 200mg/L, and samples were further incubated at 28°C. All run samples were run in triplicate, in five batches (corresponding to the 3rd, 5th, 7th, 12th and 15th day) for each microbial strain. After incubation period, for each batch, the microbial cells were removed by filtration, filtered through 20 μm membrane filter. 3 mL of each filtrate were extracted with a syringe, and sampled in quartz cuvettes. Dye concentration in aqueous solutions was measured spectrophotometrically at 500 nm for Bemacid ROT, 370 nm for Bemacid GELB, and 590 nm for Bemacid BLAU, and the decolorisation percentage was calculated using the percentage decolorisation formula:

$$\text{Decolorisation Activity (\%)} = \frac{(\text{Initial dye concentration} - \text{Dye concentration after bio-activity})}{\text{Initial dye concentration}} \cdot 100$$

All samples were run in triplicate, and mean value was used for calculation of decolorisation activity.

### *Thin Layer Chromatography*

TLC analysis was used to asses dye purity and type of dye biodegradation products. 5 μL of each stock dye solution (200 mg/L) and biodegradation products extracted in ethyl alcohol were spotted on aluminum plate (200 × 200 mm) covered with 0.25 mm silica gel 60F254 supplied by Merck (Germany) using a micro syringe. A mixture of t-butanol: acetone: water: ammonia-(5:5:1:2) vol/vol. was used as mobile phase.

### *Laccase activity*

Enzymatic activity was determined for two microbial isolates: I2 (*T.parceramosum/T.reesei/ T.longi*) and I5 (*Polyporus squamosus*), in 7 sets, respectively after 1 day, 2 days, 3 days, 4 days, 5 days, 6 days and 15 days of cultivation. Fresh cultures were grown for 7 days in 50mL flasks, and dye concentration was set at 200mg/L for each strain. Laccase activity was assessed according to method of Desai, using guaiacol (2mM) as substrate, in sodium acetate buffer (10 mM *p*H 5.0) based on its oxidation by laccase, and spectrophotometric dosage of enzymatic activity at 450 nm, after incubation 30°C for 15' [22–23]. Reaction mixture was composed of 1 mL guaiacol, 3 mL acetate buffer and 1mL of enzyme source, which was represented by mixture of strain, dye and nutritive broth, and enzyme blank, was considered 1mL of deionized water. Enzyme activity is expressed in International Unites (IU), where 1 IU is defined as the amount of enzyme that is needed to oxidize 1 micro-mole of guaiacol/minute, and the laccase activity, in U/mL is calculated with the aid of extinction coefficient of guaiacol as follows:

$$E.A. = \frac{A \times V}{t \times e \times v}$$

where: *E.A.*= Enzyme Activity; *A* = absorbance at 450 nm, *V* = total volume of reaction (mL); *t* = incubation time (minutes); *e* = extinction coefficient (12,100 $M^{-1} \times cm^{-1}$); *v* = enzyme volume.

## RESULTS

### Thin Layer Chromatography

TLC technique was used for assessment of qualitative dye purity, which may be an important factor in decolorisation efficiency, being an easy and versatile method due to high sensitivity and good reproducibility. Regarding the purity, according to TLC, the purest was Bemacid ROT dye, compared to Bemacid GELB and Bemacid BLAU, which it seems that they present multiple isomers, visualized as 3 bands for Bemacid GELB, and 4 bands for Bemacid BLAU, respectively (figure 2,*a*). Also, TLC was run for assessment of bioaccumulated dye purity (figure 2,*b-c*), compared to control, thus resulting that the accumulated dye, by fungal biomass, is identical to the initial dye, due to resulted color and migration distance.

This aspect could have an important influence over the degradative expression of each strain, and can be correlated with higher overall residual dye concentration reduction rates of all strains on Bemacid ROT dye, due to a high purity, seconded by Bemacid BLAU and Bemacid GELB.

### UV-VIS spectrophotometry

The UV-VIS spectra of the aqueous solutions of stock dyes, recorded on 200–800 nm, showed the following maximum absorbance peaks: 500 nm for Bemacid ROT, 370 nm for Bemacid GELB and 590 nm for Bemacid BLAU. Three main peaks were observed for all dyes, the strongest absorption bands (243 nm, 277 nm and 280 nm) corresponding to the chromophore group, the diazo-group. The bands of Bemacid ROT are shifted to longer wavelengths due to extended conjugation of aromatic rings (Bemacid GELB) and polycyclic aromatic ring (Bemacid BLAU*).*

### Dye decolorisation

In the present study, the microbial strains were grown in Czapek-Dox nutritive broth, selected due to low nutritive value, forcing the selected microbial strains to use the dyes as nutrients. According the quantitative determinations of the residual dyes during incubation with selected microbial strains, significant degradation rates were detected. The results obtained with Bemacid ROT used as nutritive source in the minimal culture medium indicated strong increasing of dye decolorisation for all tested samples, with minimum of 72.77% decolorisation rate, achieved in 3rd day by the isolate I1, and maximum of 98.02% achieved by isolate I6 in the 15th day (table 1). Except the strain

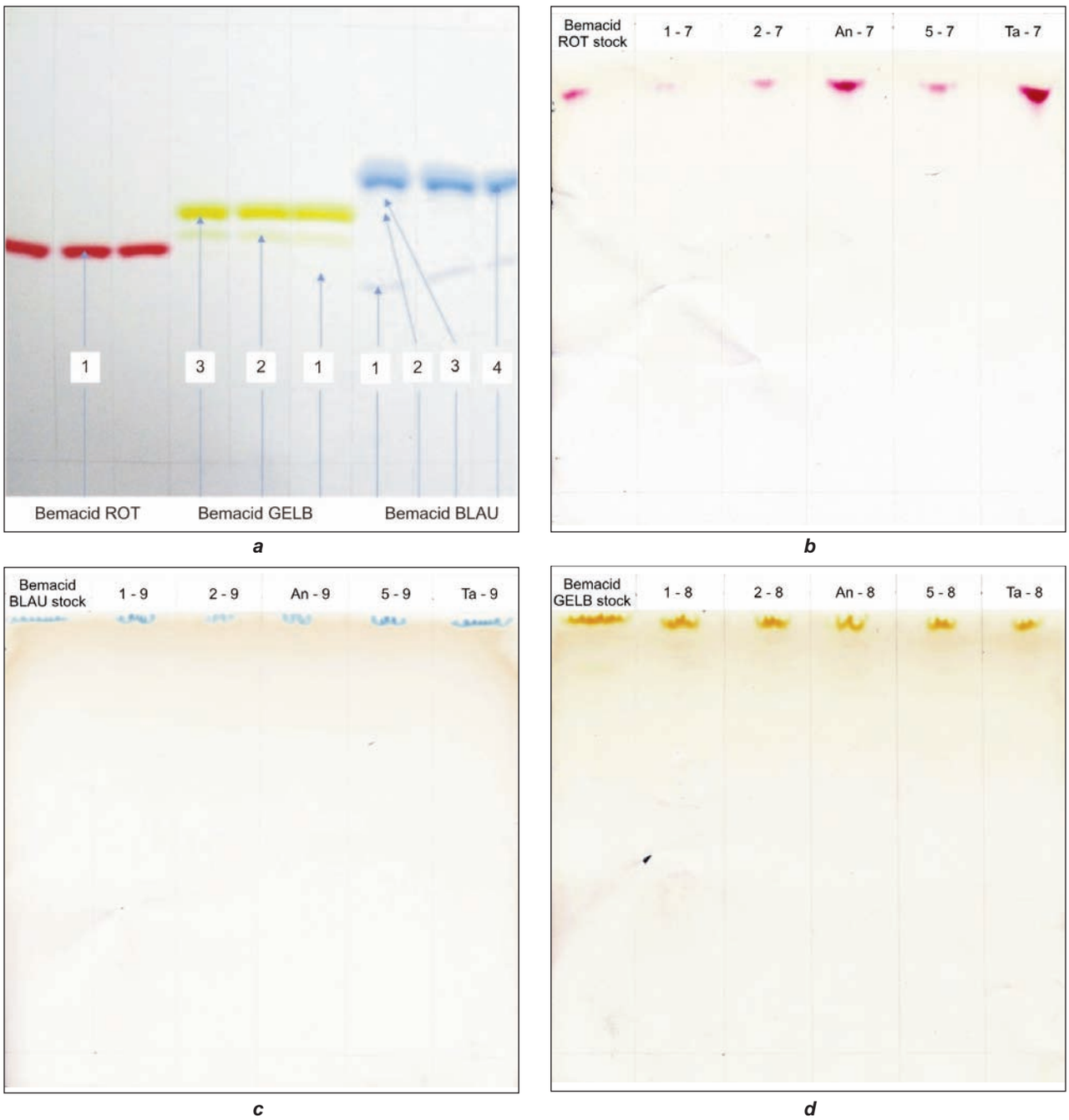


Fig. 2. Bemacid dyes TLC (the arrows indicate the possible isomers of the dyes)

Table 1

| RESIDUAL DYE CONCENTRATION REDUCTION OF SELECTED STRAINS AGAINST BEMACID ROT | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **I** | **mg/L** | **R%** | **II** | **mg/L** | **R%** | **III** | **mg/L** | **R%** | **IV** | **mg/L** | **R%** | **V** | **mg/L** | **R%** |
| **7I1** | 54.46 | 72.77 | **7I1** | 51.93 | 74.03 | **7I1** | 22.06 | 88.97 | **7I1** | 16.67 | 91.66 | **7I1** | 9.67 | 95.16 |
| **7I2** | 48.41 | 75.79 | **7I2** | 27.42 | 86.28 | **7I2** | 17.32 | 91.34 | **7I2** | 15.07 | 92.46 | **7I2** | 6.01 | 96.99 |
| **7I3** | 23.79 | 88.10 | **7I3** | 17.07 | 91.46 | **7I3** | 14.54 | 92.73 | **7I3** | 7.32 | 96.34 | **7I3** | 5.96 | 97.02 |
| **7I4** | 20.98 | 89.51 | **7I4** | 11.06 | 94.47 | **7I4** | 10.25 | 94.87 | **7I4** | 7.87 | 96.06 | **7I4** | 5.64 | 97.18 |
| **7I5** | 51.20 | 74.40 | **7I5** | 20.66 | 89.67 | **7I5** | 11.82 | 94.09 | **7I5** | 9.97 | 95.01 | **7I5** | 6.78 | 96.61 |
| **7I6** | 35.13 | 82.43 | **7I6** | 13.13 | 93.43 | **7I6** | 7.97 | 96.01 | **7I6** | 7.52 | 96.24 | **7I6** | 3.95 | 98.02 |
| **7I7** | 46.04 | 76.98 | **7I7** | 38.67 | 80.66 | **7I7** | 37.39 | 81.30 | **7I7** | 21.64 | 89.18 | **7I7** | 21.43 | 89.28 |
| **7I8** | 39.64 | 80.18 | **7I8** | 14.46 | 92.77 | **7I8** | 12.16 | 93.92 | **7I8** | 8.35 | 95.82 | **7I8** | 6.24 | 96.88 |
| **7I9** | 42.65 | 78.67 | **7I9** | 23.27 | 88.36 | **7I9** | 21.51 | 89.24 | **7I9** | 12.32 | 93.84 | **7I9** | 7.36 | 96.32 |

Table 2

| RESIDUAL DYE CONCENTRATION REDUCTION OF SELECTED STRAINS AGAINST BEMACID GELB | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **I** | **mg/L** | **R%** | **II** | **mg/L** | **R%** | **III** | **mg/L** | **R%** | **IV** | **mg/L** | **R%** | **V** | **mg/L** | **R%** |
| **8I1** | 103.44 | 48.28 | **8I1** | 86.18 | 56.91 | **8I1** | 79.65 | 60.17 | **8I1** | 54.42 | 72.78 | **8I1** | 21.27 | 89.36 |
| **8I2** | 91.40 | 54.29 | **8I2** | 84.20 | 57.9 | **8I2** | 78.20 | 60.90 | **8I2** | 50.21 | 74.89 | **8I2** | 34.81 | 82.59 |
| **8I3** | 193.58 | 3.20 | **8I3** | 179.82 | 10.09 | **8I3** | 124.83 | 37.58 | **8I3** | 55.13 | 72.43 | **8I3** | 53.95 | 73.02 |
| **8I4** | 44.38 | 77.81 | **8I4** | 36.87 | 81.56 | **8I4** | 26.97 | 86.51 | **8I4** | 15.89 | 92.05 | **8I4** | 11.86 | 94.07 |
| **8I5** | 43.50 | 78.25 | **8I5** | 36.55 | 81.72 | **8I5** | 20.77 | 89.61 | **8I5** | 17.47 | 91.26 | **8I5** | 14.50 | 92.75 |
| **8I6** | 157.55 | 21.22 | **8I6** | 108.17 | 45.91 | **8I6** | 62.84 | 68.58 | **8I6** | 39.36 | 80.32 | **8I6** | 32.99 | 83.50 |
| **8I7** | 194.97 | 2.51 | **8I7** | 147.93 | 26.03 | **8I7** | 110.74 | 44.63 | **8I7** | 53.73 | 73.13 | **8I7** | 45.55 | 77.22 |
| **8I8** | 137.67 | 31.16 | **8I8** | 83.86 | 58.07 | **8I8** | 47.97 | 76.01 | **8I8** | 30.72 | 84.64 | **8I8** | 22.71 | 88.64 |
| **8I9** | 148.96 | 25.52 | **8I9** | 50.05 | 74.97 | **8I9** | 26.92 | 86.53 | **8I9** | 25.43 | 87.28 | **8I9** | 7.87 | 96.06 |

Table 3

| RESIDUAL DYE CONCENTRATION REDUCTION OF SELECTED STRAINS AGAINST BEMACID BLAU | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **I** | **mg/L** | **R%** | **II** | **mg/L** | **R%** | **III** | **mg/L** | **R%** | **IV** | **mg/L** | **R%** | **V** | **mg/L** | **R%** |
| **9I1** | 163.41 | 18.29 | **9I1** | 146.66 | 26.66 | **9I1** | 136.47 | 31.76 | **9I1** | 107.62 | 46.30 | **9I1** | 107.39 | 46.19 |
| **9I2** | 117.02 | 41.49 | **9I2** | 34.31 | 82.84 | **9I2** | 23.87 | 88.06 | **9I2** | 17.14 | 91.43 | **9I2** | 10.54 | 94.73 |
| **9I3** | 156.66 | 21.67 | **9I3** | 54.33 | 72.83 | **9I3** | 12.45 | 93.77 | **9I3** | 11.82 | 94.09 | **9I3** | 11.31 | 94.34 |
| **9I4** | 35.68 | 82.16 | **9I4** | 35.29 | 82.35 | **9I4** | 18.07 | 90.96 | **9I4** | 8.66 | 95.67 | **9I4** | 4.36 | 97.82 |
| **9I5** | 52.01 | 73.99 | **9I5** | 45.20 | 77.40 | **9I5** | 17.17 | 91.41 | **9I5** | 16.28 | 91.86 | **9I5** | 12.46 | 95.67 |
| **9I6** | 86.58 | 56.71 | **9I6** | 35.61 | 82.19 | **9I6** | 10.46 | 94.77 | **9I6** | 9.94 | 95.03 | **9I6** | 3.23 | 98.38 |
| **9I7** | 55.27 | 72.36 | **9I7** | 51.36 | 74.32 | **9I7** | 32.07 | 83.96 | **9I7** | 23.81 | 88.09 | **9I7** | 12.40 | 93.80 |
| **9I8** | 39.55 | 80.22 | **9I8** | 32.39 | 83.80 | **9I8** | 13.65 | 93.17 | **9I8** | 12.82 | 93.59 | **9I8** | 10.41 | 94.79 |
| **9I9** | 47.23 | 47.23 | **9I9** | 53.89 | 73.05 | **9I9** | 69.26 | 65.37 | **9I9** | 10.06 | 94.97 | **9I9** | 8.76 | 95.62 |

I7, all the other isolates presented rates of decolorisation over 95%.

Different rates of dye reduction were observed when Bemacid GELB was used as nutrient during the incubation with the selected microorganisms: the results indicated lower values comparing with Bemacid ROT (table 2). Only three out of the isolates were able to induce decolorisation rates over 92%: the isolates I4 (94.07%), I5 (92.75%) and I9 (96.06%).

With Bemacid BLAU as substrate, the selected microbial strains presented different abilities to induce the decolorisation. The degradation process began more difficult for at least half of the microorganisms (isolates I1, I2, I3, I6 and I9) (table 3) but after 6 days of incubation the decolorisation rates were increased over 72% (except the isolate I1). The final results are similar with those run on Bemacid ROT, as strain I1 recorded the lowest decolorisation percentage (46.19%) and strain I6, the highest (98.38%).

Based on these results it could be assumed that the best microbial strains, for ability of decolorisation against all the dyes tested, were the strains designated as I4 (*A.niger)*, I5 (*P.squamosus*) I9 (*T.atroviridae)* and I2 (belonging to *Trichoderma parceramosum/T.reesei/T.longi* group). However, significant degradative properties were also detected in the other strains, at least against Bemacid BLAU and Bemacid ROT. The mechanisms involved in the dyes decolorisation process realized by microorganisms are not clear. Among the possible mechanisms of degradation the most popular are *p*H variation in the culture medium, the accumulation of the dyes in the cells, and the enzymatic activity (intracellular or extracellular enzymes). For this reason, the possible mechanisms involved in the decolorisation activities of the isolate I2 and I5 were analyzed.

The accumulation of the dyes in the microbial cells was observed for the strains I1, I2, I3, I5 and I6, at least for some of the dyes (Bemacid BLAU and Bemacid ROT), proving that cellular mechanisms could be involved in the dye degradation process.

Laccase is a cooper-based polyphenol oxidase enzyme that can show activity on a variety of substrates, and uses molecular oxygen as electron acceptor [24]. Bioremediation of environmental pollutants agents became an active field of laccase exploitation as a biotechnological tool of removal of toxic compounds through oxidative enzymatic coupling of contaminants [28]. Laccase enzyme is able to oxidize both phenolic and non-phenolic compounds and lead to mineralization of synthetic dyes, rendering the

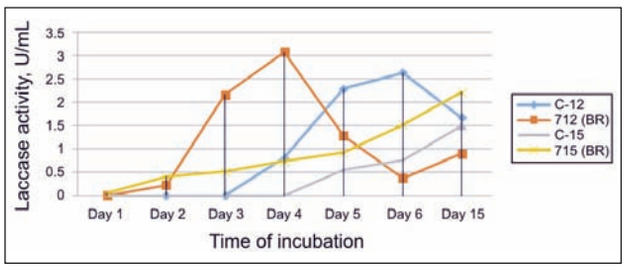


Fig. 3. Laccase activity of I2 and I5 microbial strains with (7) or without Bemacid Rot

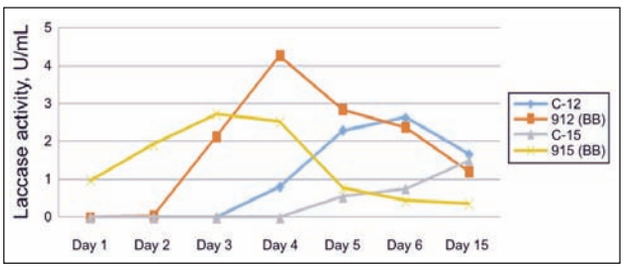


Fig. 5. Laccase activity variation for I2 and I5 + Bemacid BLAU, compared to control

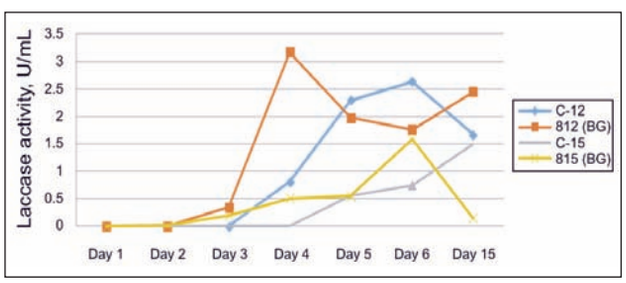


Fig. 4. Laccase activity variation for I2 and I5 + Bemacid GELB, compared to control

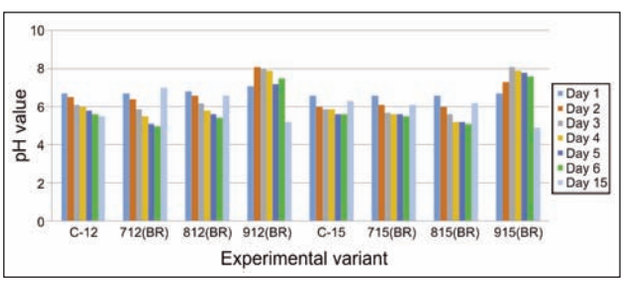


Fig. 6. *p*H variations of microbial cultures in the presence or absence of the dyes

enzyme as a valuable option for efficient, cost effective and environmental friendly bio-treatment method [29–30]. Fungi are important producers of laccase, compared with bacteria, the most important laccase producer strains belonging to white rot fungi [25–26]. Among the fungi able to produce large amounts of laccase are the macromycetes, reason for which *Polyporus squamosus* strain I5 was selected in assessment of the laccase activity, together with the strain I2 (belonging to *Trichoderma parceramosum/ T.reesei/T.longi* group) [27]. The laccase activity registered in dye containing samples was compared with control variants (without dye), designated as C-I2 and C-I5, respectively. I2 and I5 microbial strains were previously cultivated in 50 mL culture medium, for 7 days, at 28°C; at the end of the cultivation period, the suspensions were combined with each dye (to a final concentration of 200 mg/L). Differences between microbial strains incubated with the dyes, regarding the enzymatic activity determined at 1, 2, 3, 4, 5, 6 and 15 days of incubation, were observed, comparing with the control variants.

In samples with Bemacid ROT, the laccase activity of the strain I2 increased up to the 4$^{th}$ day, with a maximum of 3.08 U/mL, while the enzymatic activity of the strain I5 increased constantly, with a maximum value at the end of the incubation period (2.22 U/mL), comparing with the controls: maximum of 2.64 U/mL for I2 strain and 1.50 U/mL for I5 (figure 3).

When Bemacid GELB was used as nutrient in the culture medium, the laccase activity of the I2 strain presented similar variations in time as with Bemacid Rot, with a maximum of 3.17 U/mL in the 4$^{th}$ day. The enzymatic activity of the strain I5 was significant lower: the maximum level was detected in the 6$^{th}$ day (1.59 U/mL), followed by a massive drop of activity after 15 days of incubation (figure 4).

In the presence of Bemacid BLAU, figure 5, similar laccase activity variations for the strain I2 were observed, with a maximum of 4.27 U/mL in the 4$^{th}$ day (figure 5). However, differences were detected in I5 strain: the highest value of the laccase activity was registered on the 3$^{rd}$ day (2.73 U/mL), then the activity progressively decreased from day 5 to day 15.

It is known that the *p*H modification of aqueous solutions containing dyes could induce the color modification. For this reason, the *p*H variation during the microbial cultivation in the presence of the dyes was measured and compared with control samples (without dyes) (the initial *p*H of Czapek-Dox broth was 6,8) (figure 6).

It was shown that the dye addition shifted the *p*H values towards the neutral-slightly basic values, respectively 7.45 for Bemacid ROT, 7.34 for Bemacid GELB and 7.27 for Bemacid BLAU. During the incubation period, the overall trend of the *p*H was decreasing, except the samples containing Bemacid Blue: both microbial suspensions exhibited high values of *p*H during the first six days of incubation. However, at the end of the incubation the *p*H values were similar to the control. These results suggest that the decolorisation of the dyes used in experiments is due to other mechanisms than *p*H variation. However, it is possible that the *p*H variations to support other biological mechanisms of degradation.

## CONCLUSIONS

Bemacid group N dyes comprises acid dyes with high exhaust properties in neutral medium, good combinability, high build-up properties, good level of wet fastness, good masking of bareness due to kinetic

differences within the substrate and rapid fixing under saturated steam conditions, and are widely used in dyeing of PA clothing, sportswear and technical textiles. Decolorisation assays of tested solutions with the aid of living fungi yielded great efficiency by adsorption mechanisms and enzymatic induced processes, which catalyze dye bioremediation stages [31]. Tested fungal strains proved great efficiency in degradation of Bemacid azo-dyes, with best mean of dye residual concentration reduction in aqueous solutions of 95.94% for Bemacid ROT, seconded by Bemacid BLAU, with 90.14% and Bemacid GELB, with 86.35%. Enzymes are widely used in textile fibers modifications [32], including laccase enzymatically treatment of cotton fibers [33]. Laccase enzyme mediated biotechnological processes can be correlated with biological viable alternatives for wastewater effluents treating, which effectively combines both adsorption-biosorption mechanism, and enzymatic treatment of effluents.


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