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DISCOLOURING AND BIOREMEDIATION OF SYNTHETIC TEXTILE DYES BY WASTEWATER MICROBIAL ISOLATES

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Abstract

Fungal biomass has the ability to decolorize textile industry wastewaters by a series of bio-accumulation/bio-sorption mechanisms, posing an economically promising, eco-friendly and feasible alternative to conventional methods. The present study explored the qualitative potential of previously isolated fungal strains from samples of post-finishing textile wastewater in treatment of synthetic dyes solutions based on Bemacid azo-dyes (red, yellow and blue). The analyses were carried out on solid nutritive media with 200g/L dye concentration, for 9 microbial strains. Previously isolated fungal strains were identified by ITS-RFLP method. Qualitative assessments revealed the increased tolerance of microbial isolates to textile azo-dyes, and their capacity to degrade into non-colored intermediates.

Key words: *textile dyes, wastewater, fungi, bioremediation.*

INTRODUCTION

There is a real actual concern regarding dyestuff effluents entering ecosystems, with both national and international regulations for industrial wastewater requiring significant elimination of dyestuff content from the effluents. None of actual effluent treatment methods are sufficiently effective in disposing of typical dyestuff, modern techniques including chemical methods (coagulation / flocculation, activated carbon, adsorption and bio-treatment, ozonation, sodium hypochlorite treatment, photochemical decolourization).

Textile dyes are usually designed to withstand microbiological fading. Usually, dyes concentrations range from 10 to 200 mg/L, for intense coloration. Textile dyes have the tendency to bio-accumulate in living organisms, sustained by their slow degradation. Wastewater recycling represents a viable solution to increase textile industry technologies sustainability, reducing pollutants impact on the environment (X. Lu al., 2010). Textile industry is one of the largest water consumers, due to numerous operations involved in textile processing and finishing, which involve high volumes of water, resulting in dynamic polluting vectors (C. Allegre al., 2006). More than 25% of wastewater resulted from textile and paper industry activities are being released into main water effluents (Shaul al., 1991).Industrial effluents contamination leads to inevitable alteration of pH value, increases biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and leads to intense colorations (Dutta al., 2002; Fang al., 2004; Asad al., 2007), leading to water sources that pose toxic, mutagenic and/or carcinogenic threat for water microbial populations and animals (Gunasekaran al., 2006).

Azo-dyes represent a large class of synthetic dyes used in textile industry, with more that 50% of commercial dyes used in industry belonging to this class. Azo-dyes contain nitrogen as the azo group −N=N− as part of their molecular structures. Most azo dyes contain only one azo group, but some contain two (disazo), three (trisazo) or more. In some conditions, azo-dyes can change structure, resulting in carcinogenic and allergenic aromatic amines. Conventional wastewater

treatment methods, such as coagulation, filtration and chemical flocculation (Gogate al., 2004), based on physical-chemical principles are expensive methods which lead to additional disposable compounds, compared to microbial degradation and bioremediation, with lower costs and environmental impact (Verma al., 2003; Mendez-Paz al., 2005; Pandey al., 2007). Bioremediation of azo-dyes contaminated wastewater can be possible with the use of bacterial strains, such as: *Bacillus cereus, Pseudomonas putida, Pseudomonas fluorescence* (Khehra al., 2005), *Pseudomonas desmolyticum* and *Bacillus sp.*(Kalme al., 2009).

MATERIALS AND METHODS

Fungal isolates

Fungal strains used in this study were previously isolated from a source of wastewater resulted from textile treating and finishing stages. The isolates were plated on 4 synthetic medias: Potato-Dextrose-Agar (PDA), Sabouraud-Agar, Malt-Agar (MA), Czapek-Dox. A total of 9 strains were used in the experiments, from which 8 strains were unknown, and subjected for identification, and 1 collection strain of *Aspergillus niger* IMI 45551.

DNA extraction and PCR fingerprinting

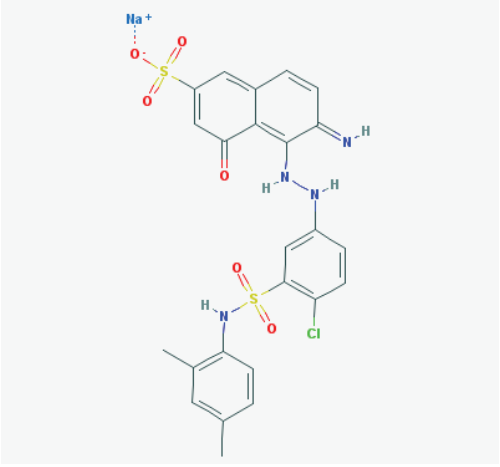
DNA isolation from microbioal strains (new isolates and collection strains) was performed using the method of Raeder and Broda (1985) with slight modification (the cells lysis was optimized by using glass beads and a Mini-Beadbeater BioSpec). For DNA amplification primers for the ITS region of the nuclear small-subunit rRNA gene were used: ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC)(White et al., 1990). The PCR reactions were performed in a total volume of 25 μL, containing 1x standard PCR incubation buffer, 0.5 μM of each primer, 0.2 mM of each of the four dNTPs, 0.025 Uμl^{-1} of DreamTaq polymerase and 30 ng genomic DNA. The PCR conditions are: 94°C for 45 sec, annealing at 55.5°C for 45 sec, 60 sec elongation at 72°C and final extension of 10 min at 72°C in 34 cycles. A negative control with all the reaction mixtures except the DNA template was included with each set of the PCR amplification Reactions (Kamala et al., 2015). For restriction fragment analysis, PCR products were cleaved with five restriction enzymes, namely HaeIII, HinfI, HhaI, MseI and SduI., in 16 μL reaction mixtures consisting of: 10x buffer (2.0 μL), enzyme (1 μL), PCR product (10 μL), and pure water (3 μL). Reaction mixtures of HaeIII, SduI, HinfI and HhaI enzymes were incubated for 2 h at 37°C, and 65°C for MseI. The restriction DNA fragments were separated in 2.0% (w/v) agarose gel electrophoresis, and the visualized in UV light after staining with ethidium bromide.

Nutritive substrate

Synthetic nutritive media was used for qualitative assessment of fungal isolates efficiency, with the following composition: 1g/L K_2HPO_4, 0.01g/L $ZnSO_4$, 0.05g/L $CuSO_4$, 0.5g/L $MgSO_4$, 0.01g/L $FeSO_4$, 0.5g/L KCl, 3g/L $NaNO_3$, 10g/L glucose and 20g/L of agar. The media was sterilized for 15' at 121^{o}C, with post sterilization pH value of 5.5.

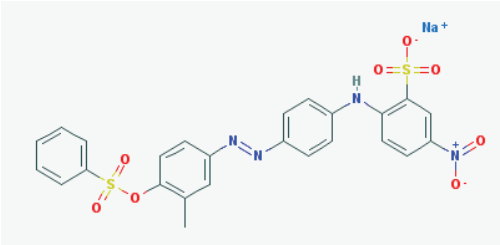
Textile dyes

3 industrial textile Bemacid acid dyes from Bezema, for polyamide and wool were tested: Bemacid ROT N-TF (Figure 1), Bemacid GELB N-TF (Figure 2) and BEMACID BLAU N-TF (Figure 3). Used dye concentration was of 200mg/mL for each dye.



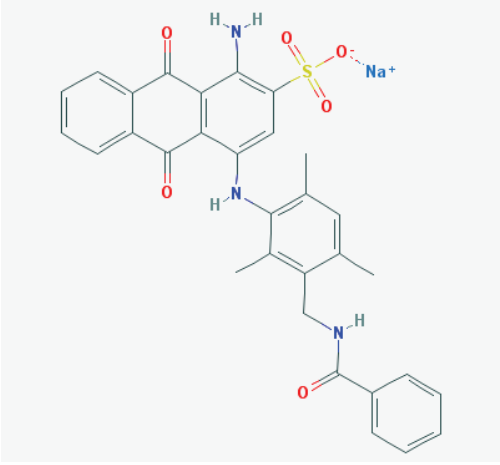
sodium 6-amino-5-((4-chloro-3-(((2,4-dimethylphenyl)amino)sulphonyl)phenyl)azo)-4-hydroxynaphthalene-2-sulphonate

Figure 1. Bemacid Rot N-TF structure



sodium 6-amino-5-((4-chloro-3-(((2,4-dimethylphenyl)amino)sulphonyl)phenyl)azo)-4-hydroxynaphthalene-2-sulphonate

Figure 2. Bemacid Gelb N-TF structure



sodium 1-amino-4-({3-[(benzoylamino)methyl]-2,4,6-trimethylphenyl}amino)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate

Figure 3. Bemacid BLAU N-TF structure

RESULTS AND DISCUSSIONS

In this study, it was evaluated the discoloring potential of 9 fungal isolates when used against Bemacid dyes, immobilized in solid nutritive media. Each dye was used in concentration of 200mg/L, and the fungal strains were inoculated in the middle of the plates, and incubated for 16 days, in darkness, room temperature. After the incubation period, the Petri plates were visualized for signs of decolorization and/or dye bioaccumulation by the microbial biomass (Figure 6, 7 and 8)

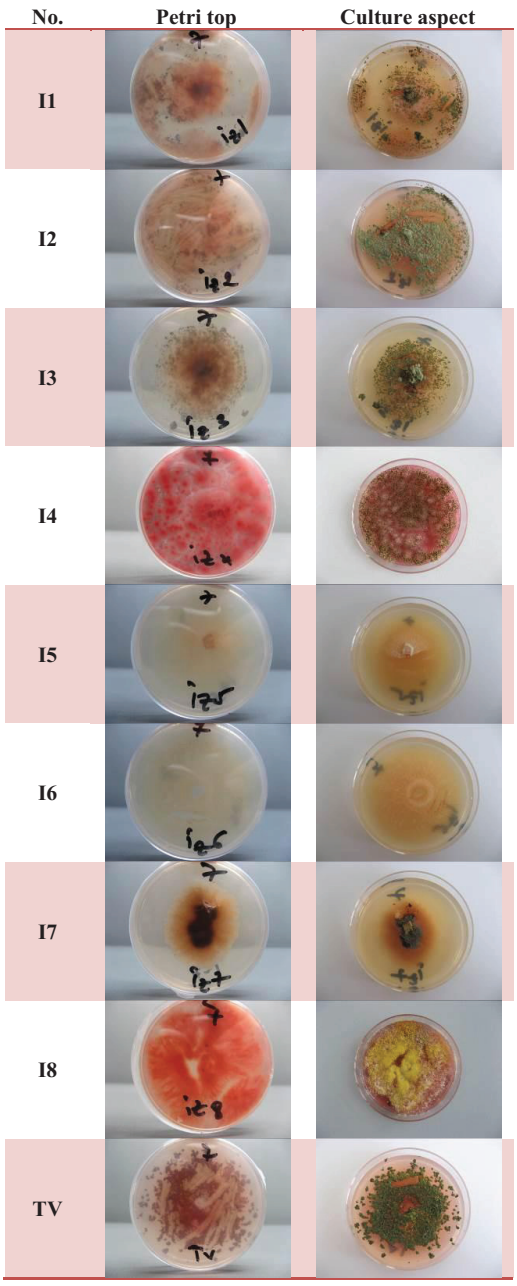


Figure 6. Bemacid ROT vizual discoloration

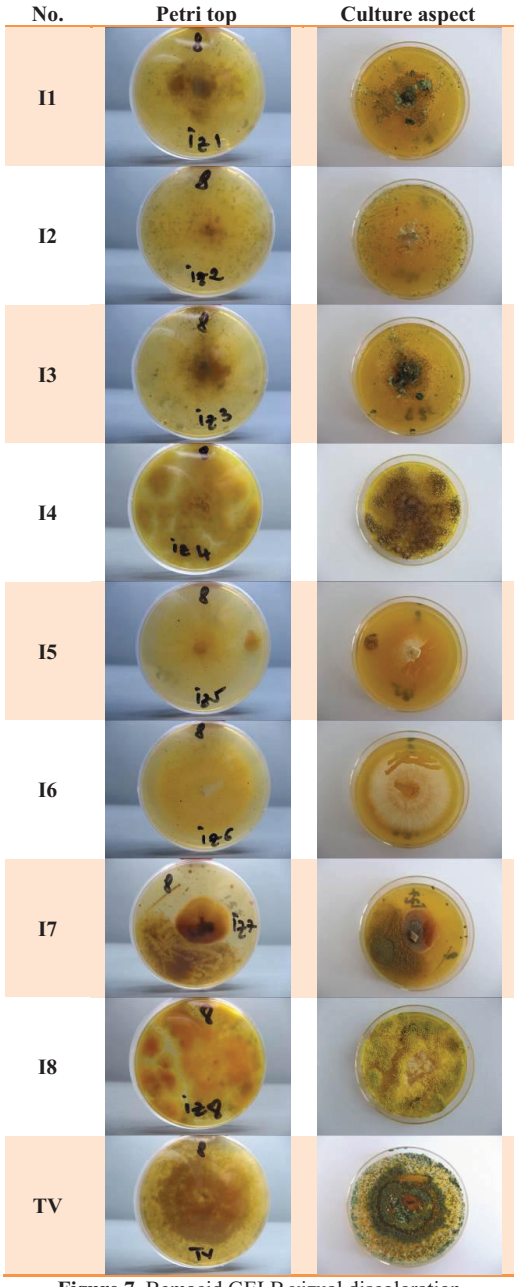


Figure 7. Bemacid GELB vizual discoloration

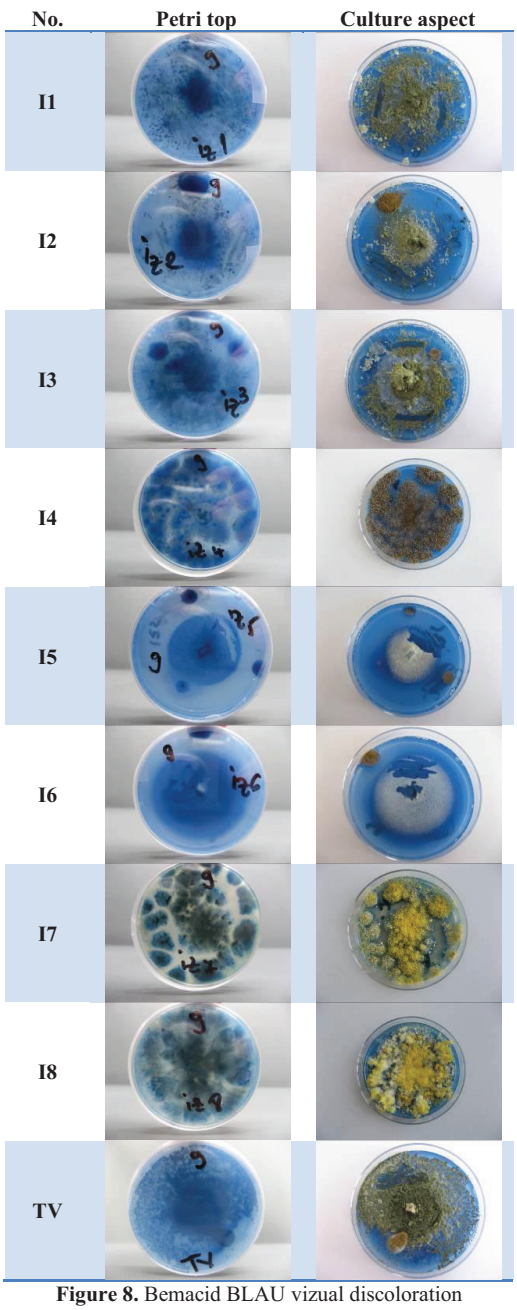


Figure 8. Bemacid BLAU vizual discoloration

Molecular identification

A total of 8 microbial strains, new isolates and collection strains were used in experiments for species identification by ITS-RFLP method. Differences in the length of the amplicons obtained after PCR with primers for ITS1/ITS4 regions were detected after agarose gel electrophoresis (Figure 4).

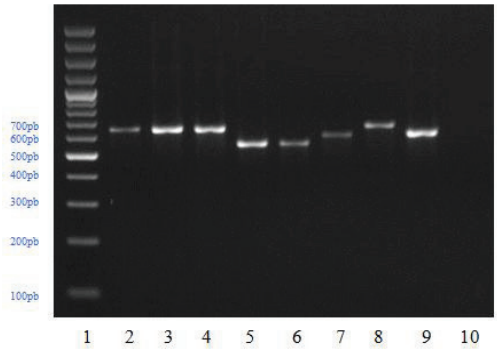


Figure 4. Amplicons obtained with ITS1/ITS4 primers. 1= ladder DNA; 2 – fungal strain 1; 3 = fungal strain 2; 4 = fungal strain 3; 5 = *Polyporus squamosus*; 6 = *Fusarium oxysporum*; 7 = yeast strain 7; 8 = yeast strain 8; 9 = *Trichoderma spp*.; 10 = no DNA

In order to differentiate the strains, restriction analysis of the PCR fragments was conducted, comparing with the data from literature. Comparing the isolates 7 and 8, the restriction profiles (Table 1) were different comparing with several yeast strains used as reference (*Sacharomyces cerevisiae, Yarrowia lipolytica, Metschnikowia pulcherima, Rhodotorula spp.*) (data not shown) and didn't allowed the identification of the species.

Table 1. Restriction profile of the isolates 7 and 8

Strains	Restriction fragments length (bp)		
	*Hha*I	*Hae*III	*Hinf*I
7	100+230+300	210+400	80+210+340
8	70+100+160+200+220	50+190+320	70+140+190+340

When the comparison was realized between the strains 1, 2, 3 and TV (*Trichoderma spp*), the restriction profiles with the enzymes HhaI, SduI and Hae III were similar for the strains 1-3 and with small differences for TV strain (Figure 5).

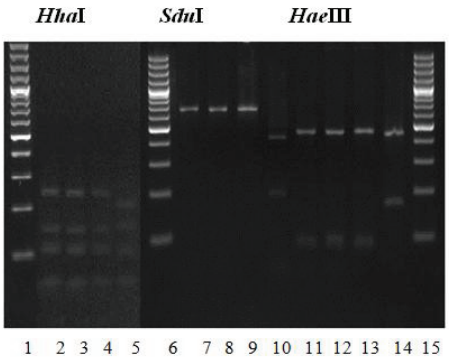


Figure 5. Electrophoretic profile of the restriction fragments obtained with HhaI, SduI and Hae III restriction enzymes. 1, 6, 15 – ladder DNA; 2, 7, 11 – fungal strain 1; 3, 8, 12 – fungal strains 2; 4, 9, 13 – fungal strain 3; 5, 10, 14 - TV

Based on data from literature it is possible that the new isolates could belong to *T. parceramosum / T. reesei / T. longi* group, and TV strain to *Trichoderma atroviride* (Table 2) but further analysis, including the sequencing of PCR products are necessary for confirmation.

Table 2. Restriction profile of the isolates 1, 2, 3 and TV

Strains	Restriction fragments length (bp)		
	*Hha*I	*Sdu*I	*Hae*III
1	110+150+250	630	70+90+480
2	110+150+250	630	70+90+480
3	110+150+250	630	70+90+480
TV	110+160+210	190+430	170+450

Clear identification was obtained for the strains *Fusarium oxysporum* and *Polyporus squamosus* using ITS-RFLP analysis with *Sdu*I, *Hae*III, and *Mse*I restriction enzymes (Table 3).

Table 3. Restriction profile of the strains of *F.oxysporum* (6) and *Polyporus squamosum* (5)

Strain	Restriction fragments length (bp)		
	*Sdu*I	*Hae*III	*Mse*I
5	540	90+110+340	540
6	540	90+110+340	50+90+370

CONCLUSIONS

The results of the present study indicate that the tested strains can yield well to great discolouring potential. Either by enzymatic cleavage of textile azo-dyes into non-coloured intermediates, or by bioaccumulation, the microbial

strains show effective decolourization of solid media immobilized dyes.

The molecular techniques are valuable for genetic analysis of microbial strains and could allow the identification of the species. However, standard strains are necessary in order to perform a precise analysis.

Future work will be concentrated on quantitative assessment of degradation of azo-dyes with the use of fungal isolates, in liquid media, and spectrophotometric dosage of reduction rates.

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