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CHROMATOGRAPHIC CHARACTERIZATION OF BEMACID ROT DYE COMPOUNDS FOLLOWING *CERIOPORUS SQUAMOSUS* BIOREMEDIATION

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Abstract: *Fungi mediated bioremediation of industrial wastewaters containing azo-dyes has been gaining increased attention in the last years. One of the downsizes of bioremediation of azo-dyes is the possibility of resulting degradation aromatic amines, following breakage of azo-bond by microbial azoreductase. In the present study, HPLC technique was used for the analysis of Bemacid Rot (Bezema) azo-dye compounds, resulting from treatment of synthetic-dyed wastewater with Cerioporus squamosus fungal strain. C. squamosus is a basidiomycete bracket fungus, that has gained little attention towards bioremediation methods of industrial wastewaters. Chromatographic analyses were performed on three experimental sets: solutions of pure dyes dissolved in distilled water at concentration of 200 mg/L; solution of sample resulting from the biodegradation of dyes (containing nutrients from the culture media: salts, fungal cells, dye, biodegradation products of different polarities) and on a solution of the dye dissolved in the nutritive media. Analyses were conducted at five wavelengths (226nm, 243nm, 304nm, 500nm and 550nm). Major differences could be highlighted, in terms of elution profiles, retention times, number of peaks, and proportion of components. This allowed the highlighting of dye degradation compounds with lower retention times, which can indicate the degradation of the azo-dye into highly polar compounds.*

Key words: *Moving bed biofilm reactor, fungi, wastewater, azo-dyes*

1. INTRODUCTION

Water in the textile industry is characterized by large fluctuations in physical-chemical parameters [1] such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, conductivity, turbidity, color and salinity, their composition depending on both the content in organic compounds and the type of dyes used in the finishing processes [2].

The toxic effect of azo-dyes can be the result of their direct action or amine derivatives resulting from azoic biotransformation [3-4]. The color of the textile effluents is notable at a concentration of about 1 mg/L, their average concentration in the textile effluents being about 300 mg/L, with a loss of dyes in the dyeing processes, ranging from 2-60% [5]. The presence of

industrial dyes in water effluents reduces the pathway of solar rays, with difficulty in lower volumes, with effect on photosynthetic activities of the aquatic flora, decreasing of dissolved oxygen concentration, with negative effects on aquatic flora and fauna [6].

Biological treatment methods of industrial wastewater are often presented as less costly alternative methods, compared to conventional physical-chemical ones. Technological processes of biodegradation by fungi are starting to be used in degradation of pollutants by adsorption on viable or inactive biomass, use of microbial biomass as biosorbents, microbial bioremediation systems, all of which have been successfully applied in industrial effluent treatment technologies [7][8].

2. MATERIALS AND METHODS

2.1 HPLC analysis

High Performance Liquid Chromatography analysis was carried out on an Agilent Series 1100 spectrophotometer, with quaternary pump and MWD detector (with multiple wavelengths). The process parameters were as follows: Phenomenex-Kinetex C18 100µ 2.6µm column, thermostated at 250°C; 70% CH_3OH/30% HOH, vol / vol, mobile phase; 10µl injection volume; 0.7 ml/min flow rate; 2 minutes of post-time (wash time after each analysis); 260 bar maximum pressure during column analysis. Dye solutions and samples were filtered on filter paper and subsequently on 20µm porosity filters, after which they were analyzed on the spectrophotometer. The wavelengths at which dye separation was carried out was selected starting from previous UV-VIS analysis, for Bemacid Rot dye, with absorption maximum at 500nm, and two secondary peaks at 243nm and 304nm.

2.2 Azo-dye and strain

The chromatographic analysis was carried out on Bemacid Rot azo-dye (Fig. 1), from BEZEMA AG Company (N-TF (CAS EINECS: 276-115-7), $C_{24}H_{20}ClN_4NaO_6S_2$, M = 583.0 g/M). Dye degradation was induced by enzymatic activity of *Cerioporus squamosus* fungal strain, grown in liquid nutritive broth (Czapek-Dox: 30g/L sucrose, 3g/L sodium nitrate, 0.5g/L magnesium sulfate, 0.5g/L potassium chloride, 1.0g/L potassium phosphate dibasic, 0.01g/L ferrous sulfate, pH 7.3 at 25oC) incorporating the azo-dye for supplying nutrients (data not shown in this paper). Selected microbial strain, *Cerioporus squamosus*, is a basidiomycete bracket fungus, belonging to the Basidiomycota phylum (Agaricomycetes class), with saprobic activity on decaying hardwood logs and stumps.

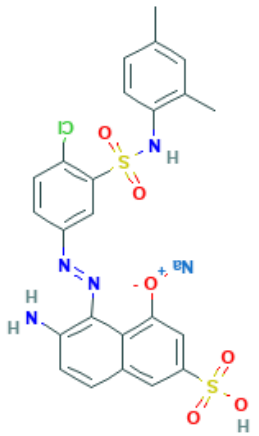

***Fig. 1**: Bemacid Rot chemical structure*

3. RESULTS AND DISCUSSIONS

For separating and identifying the components of the mixture resulting from the degradation of the dyes, High Performance Liquid Chromatography (HPLC) was used due to high accuracy, high separation efficiency and relatively simple sample preparation.

In Fig. 2 are shown the chromatograms of Bemacid ROT dye at 200 mg/L, at 226nm, 243nm, 304nm, 500nm and 550nm wavelengths.

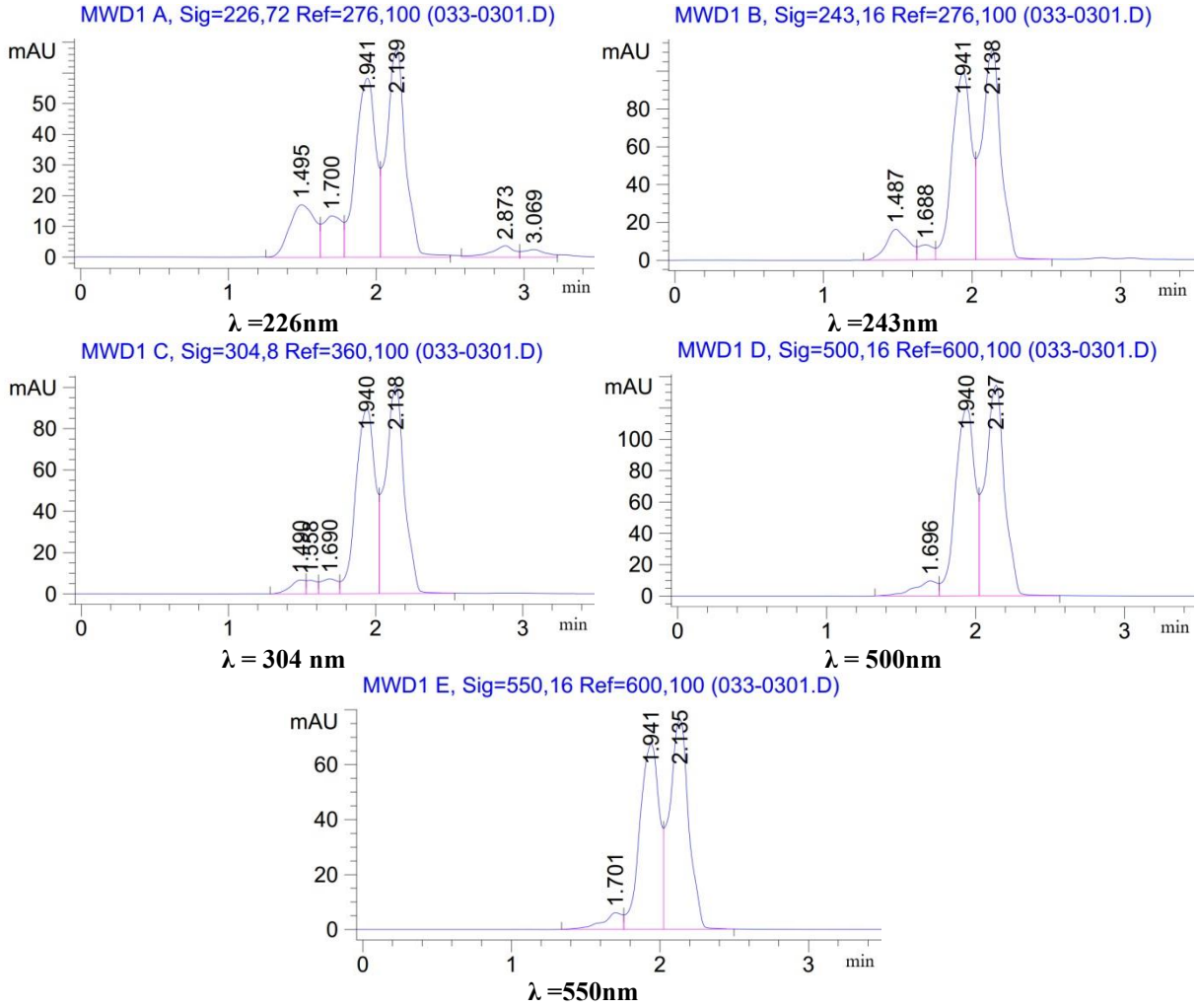

***Fig. 2**: HPLC-MWD chromatogram at 200mg/L Bemacid Rot dye*

Results show that Bemacid Rot dye has a different number of components. Thus, at λ=226nm there are 5 components, at λ=243nm, 4 components, at λ=304nm, 5 components, at λ=500 and at λ=550nm there are 3 components. Taking into account the peak integration area, it can be observed that at all wavelengths there are two major peaks at a retention time of approximately 1,941 and 2,135-2,138 minutes, representing 45% and 50% of the total integration area.

Furthermore, after bioremediation method (data not shown), the obtained solutions were chromatographically analyzed at 226nm, 243nm, 304nm, 500nm and 550nm wavelengths (Fig. 3).

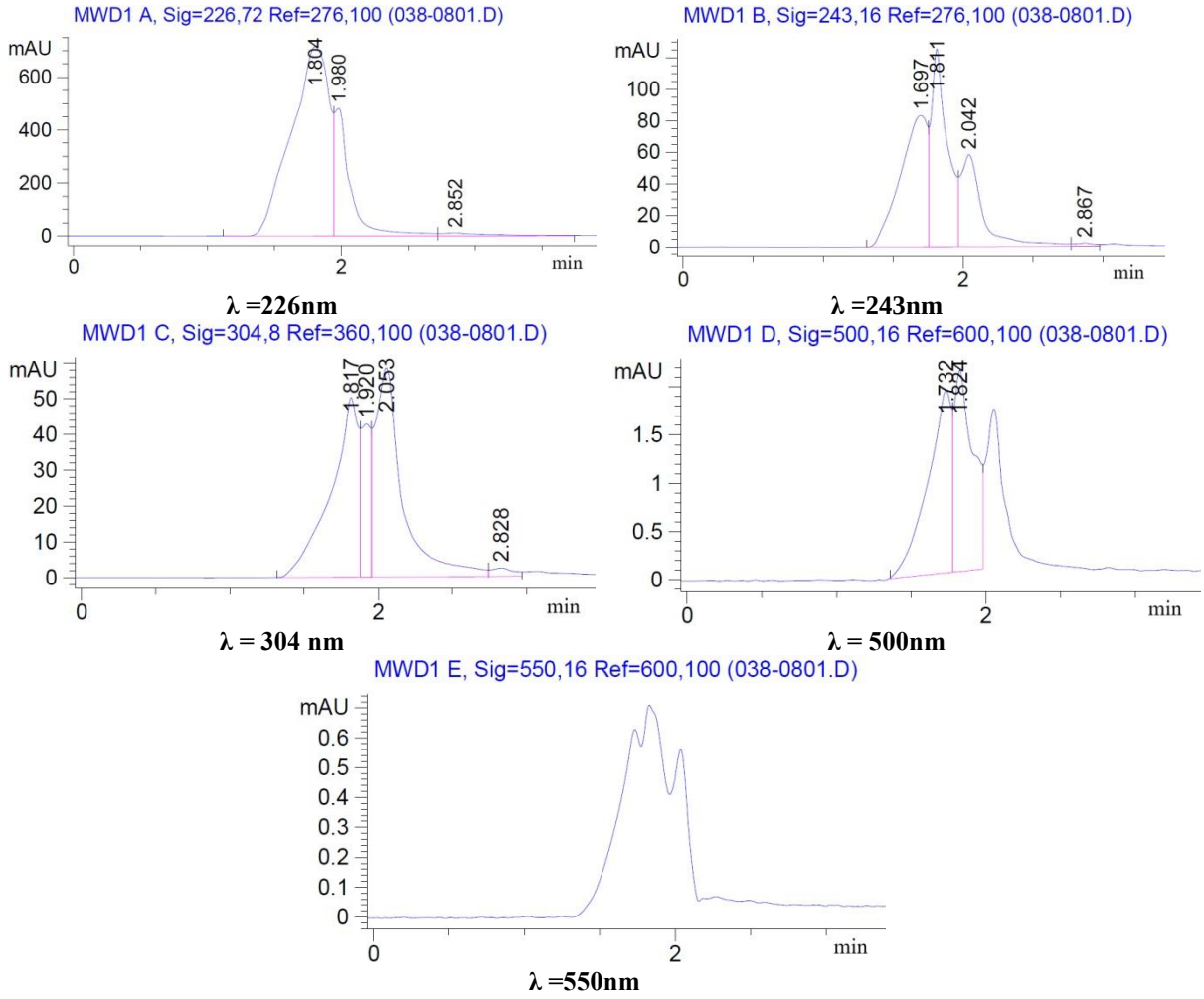

***Fig. 3**: HPLC-MWD chromatogram of sample R5 (Polyporus squamosus)*

Analysis shows major differences in elution profile, retention times, number of peaks, and proportion of components, when compared to the chromatograms of initial dye solution. It is noted the formation of dye degradation compounds with lower retention times, which cand lead to the hypothesis of dye degradation into highly polar compounds.

Furthermore, for assessment of influence of nutritive media composition on the analysis, and in order to remove the resulting compounds from the reaction media (enzymes, proteins, amino acids, sucrose, sodium nitrate, di-potassium sulfate, potassium chloride, ferrous sulphate, magnesium sulphate), chromatography analysis (at 226nm, 243nm and 304nm, 500nm and 550nm) was performed under similar conditions to those of Bemacid Rot and samples subjected to biodegradation (Fig 4 and Tab. 1).

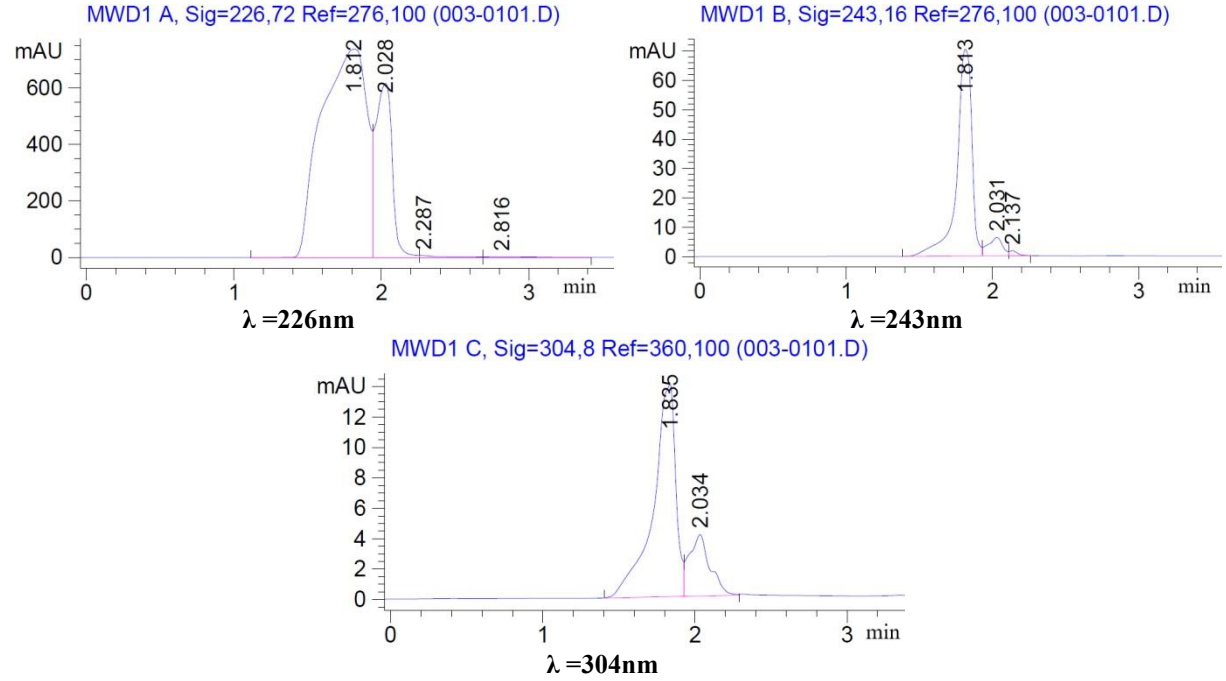

***Fig. 4**: HPLC-MWD chromatogram of Bemacid Rot and culture media*

***Table 1**: Culture media HPLC-MWD chromatogram characteristics*

Pic	λ=226nm		λ=243nm		λ=304nm	
	Ret. (min)	Area (%)	Ret. (min)	Area (%)	Ret. (min)	Area (%)
1	1.812	75.99	1.813	90.89	1.835	78.89
2	2.028	23.27	2.031	7.87	2.034	21.11
3	2.287	0.40	2.137	1.24	-	-
4	2.816	0.33	-	-	-	-

Obtained data shows that the culture media has 4 peaks at 226nm, 3 peaks at 243nm, 2 peaks at 304nm (no data was obtained at 500 and 550nm. Comparing the results with those of analysis carried out on *C. squamosus* samples, it was observed that the peaks eluted at 1,812 minutes are also found on the chromatograms of the sample, (in similar proportions at λ=226 nm) at approximately the same elution times and % area, indicating that these peaks are from the culture medium. These peaks are also found at 243nm and 304nm, but in considerably smaller proportions.

4. CONCLUSIONS

High Performance Liquid Chromatography (HPLC) analysis allowed the separation and identification of the compounds resulting from the biodegradation of Bemacid Rot dye by *Cerioporus squamosus* fungal strain. The decrease or even disappearance of the compounds eluted from the pure dye solution could be observed, highlighting the biodegradative action of the strain or isomeric transformation of the dye. The determinations were performed at five wavelengths for each sample, so partial degradation of the dye may be evidenced, in some samples, with the formation of sub-compounds, with absorption especially in the UV region. Analysis of the proportion of peaks and elution times, generally lower, reinforced the hypothesis of dye degradation, with the formation

of more polar compounds, which determined their elution faster on the column and the degradation of the functional groups and the aromatic nuclei of the original dye.

Chromatographic analysis of the culture medium did not reveal the identification of their compounds with the chromatographic profiles of the dyes but revealed extremely low absorption rates of the dyes in the culture medium with very low integration of the signals and elution times.

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