DEGRADATION OF AZO-DYES BY VIABLE BIOMASS OF *ASPERGILLUS NIGER*

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REZUMAT. Scopul acestei lucrări a fost de a evalua capacitatea de degradare a tulpinii *Aspergillus niger,* pe trei coloranţi azoici industriali, respectiv Bemacid Blau, Bemacid Rot şi Bemacid Gelb. Degradarea coloranţilor s-a efectuat prin analiza HPLC, atât pe coloranţi in stare pură, cât şi pe coloranţii biodegradaţi şi a permis fie evidenţierea degradării coloranţilor (dispariţia totală a peak-urilor eluate) sau transformarea izomerică a acestora (schimbarea fie a intensităţii peak-urilor, fie a modificării uşoare a timpului de eluti) prin mediere microbiană.

Cuvinte cheie: bioremediere, fungi, coloranți textili

ABSTRACT. The aim of this work was to assess the degradation ability of *Aspergillus niger* strain, on three industrial textile azoic dyes, namely Bemacid Blau, Bemacid Rot and Bemacid Gelb. Dyes degradation was performed by HPLC analysis, on both pure state dyes and biodegraded dyes, and allowed either highlighting the degradation of the dyes (total disappearance of eluted peaks) or isomeric transformation (change of either peak intensity or slight modification of eluted time), by microbial mediation.

Keywords: bioremediation, fungi, textile dyes

1. INTRODUCTION

Colored effluents originating from textile industry pose great concern to both aquatic life and potable water streams. Following finishing processes, a high quantity of dyes are lost in water effluents [1], which, in anaerobic conditions, can lead to formation of toxic compounds [2]. Textile industry involves great amounts of dyes and pigments used in finishing processes, of which it is estimated that at least 10% of the dyestuff used ends up into different environmental components through waste effluents [3][4]. The most used dyes in textile industry are represented by azo-dyes, copper phthalocyanine and anthroquinone, their degradation (especially of azoic dyes) being very difficult due to complex matrix [5][6].

Currently, physical and chemical methods are widely used as treatment methods for effluent decolorization [7], such as membrane filtration, photo catalytic methods, ion exchange, precipitation, flocculation, ozonation etc. [8]. However, these methods often require high amounts of chemicals and are energy driven processes, thus leading to need of development of both efficiency alternatives and cost efficient treatment methods for colored effluents.

Biological remediation is regarded as an eco-friendly alternative to conventional treatment methods, microbial biomass being able of bio degradative driven processes through enzymatic, biosorption, adsorption processes [9]. Microbial decolorization of industrial effluents is slowly winning terrain as it is less expensive than traditional methods, eco-friendly (less generated sludge) [10]. Lately, there are numerous studies involving the biodegradative potential of *Aspergillus niger* strain for decolorization of textile industrial dyes [11][12]. Hence, in the present experimental work, the feasibility of using viable biomass of *Aspergillus niger* for textile azo-dyes was examined.

2. EXPERIMENTAL WORK

2. 1. Microbial strain

A microbial strain of *Aspergillus niger* (IMI 45551) was used in current experiments. The strain was grown in fresh batches in Czapek-Dox nutritive broth, incubated for 14 days at 28^{o}C. After incubation period, strain was grown at fermenter level (Biotec FE 007), in Czapek-Dox broth (36 hours at 28^{o}C) for high yield of microbial biomass. Obtained biomass was afterwards transferred into

1000mL flasks, with nutrient broth, and refrigerated, as stock strain.

2. 2. SEM analysis

Morphological analysis of selected strain growth was assessed by Scanning Electron Microscopy (SEM), on a Quanta 200 (Fei, Holland). Strain was grown in pure cultures, on Czapek-Dox media, for 14 days, at 28^{o}C. After incubation period, microbial biomass was scraped with a loop and deposited on carbon tape, mounted on SEM stab, without coating. Morphological analyzes were carried out on GSED detector, ESEM mode, beam spot size set to 4.0, 10kV filament voltage, with image acquisition set to 27.2 seconds.

2. 3. Textile dyes

Industrial azo-dyes were used in the degradation experiments, from Bezema AG: Bemacid BLAU N-TF(CAS EINECS: 267-224-0), C31H28N3NaO6S, M=593.63 g/M; Bemacid ROT N-TF(CAS EINECS: 276-115-7), C24H20ClN4NaO6S2, M=583.0 g/M; Bemacid GELB N-TF(CAS EINECS: 235-406-9), C25H19N4NaO8S2, M=590.56 g/M. Stock dyes solutions of 5g/L concentration were prepared, in Czapek-Dox nutritive broth, stirred at 500rpm, for 3hours as 28^{o}C, and then sterilized at 121^{o}C for 15 minutes.

2. 4. Degradation experiment

Degradation experiments were carried out in 50mL Erlenmeyer flasks, with 10g of viable microbial biomass and 2mL of each dye stock solution, for a final volume of 50mL and final dye concentration of 200mg/L. All samples were incubated for 3 days, at 28^{o}C, in static conditions. Following incubation period, the microbial cells were removed by filtration, filtered through 20µm membrane filter.

2. 5. HPLC Analysis

HPLC analysis was used for assessment of textile dyes degradation. Previously obtained filtrates were chromatographed on an Agilent 1100 Series HPLC, with quaternary pump and MWD detector (with multiple wavelengths). Test conditions were set to: column: Phenomenex-Kinetex C18 100^{a} 2.6µm, 150x4.6 mm (length and diameter), heated at 25^{0}C; mobile phase composition: 70% CH_3OH/30% HOH, vol/vol.; injection volume: 10 µl; flow: 0.7 ml/min; post-time (washing time after each analysis): 2 minutes; maximum pressure in column during analysis: 260 bar. The wavelengths at which color and sample separation were made were selected on the basis of prior UV-VIS analyzes [13]. Thus, the absorption maxima used for the dyes were: Bemacid ROT: maxima of 500nm, with 2 secondary maxima at 243nm and 304nm; Bemacid GELB: maxima of 370nm, with 2 secondary maxima at 277nm and 436nm; Bemacid BLUE: maxima of 590nm, with 2 secondary maxima at 629nm and 280nm. HPLC analysis was carried out on both the pure dyes, used as control, and on biodegraded ones.

3. RESULTS AND DISCUSSION

3. 1. SEM analysis

Strain of *Aspergillus niger* is often used in waste management and biotransformation processes, being an organism with prevalence in mesophilic environments [14]. The strain used in presented experiments is a collection strain used in a wide range of synthetic tests and industrial applications.

Microscopic analysis carried out at working pressures between 200-227 Pa allowed the highlighting of specific morphological properties, such as conidiophores and hyphae (Fig. 1).

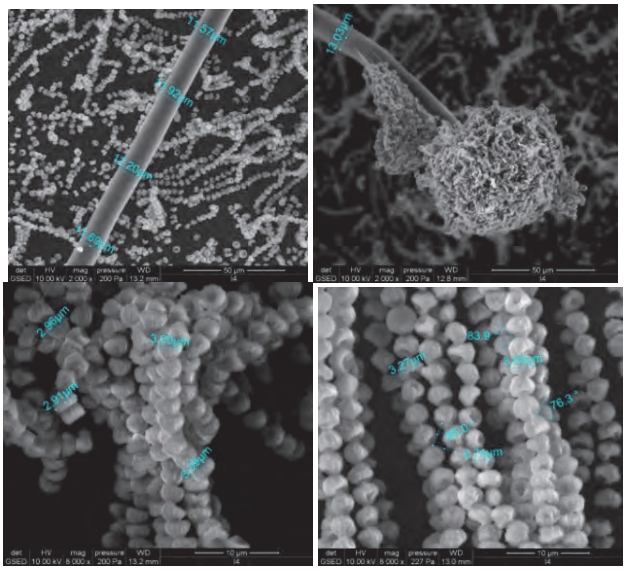

Fig. 1. SEM analysis of morphological characters for *Aspergillus niger* strain.

SEM analysis revealed the morphology of fungal spores with concave spores structures, on organized in conidiophores, highlighting the phyliades with dimensions determined between 2,42µm-3,09µm. Hyphae are tubular and smooth, with very small width dimensions, ranging from 11,57µm to 13,03µm. *Aspergillus niger* spores have large inter-spores angles, with values between 73,3^{o}-85,0^{o}.

3. 2. Dyes degradation and HPLC analysis

Experiments to reduce the residual dyes concentrations in solution highlighted discoloring abilities of *Aspergillus niger* strain, for all tested dyes (data not shown). HPLC analysis was used as a high sensitivity assay method for determining the behavior of textile industrial dyes in bioremediation treatment methods and characterization of the resulting compounds.

Figure 2 shows chromatograms of Bemacid ROT dye at 200mg/L concentration at wavelengths of 226nm, 243nm, 304nm, 500nm and 550nm. The absorption maxima of the dye, determined by UV-VIS, are at 500nm, the maximum peak, with 2 secondary peaks at 243nm and 304nm.

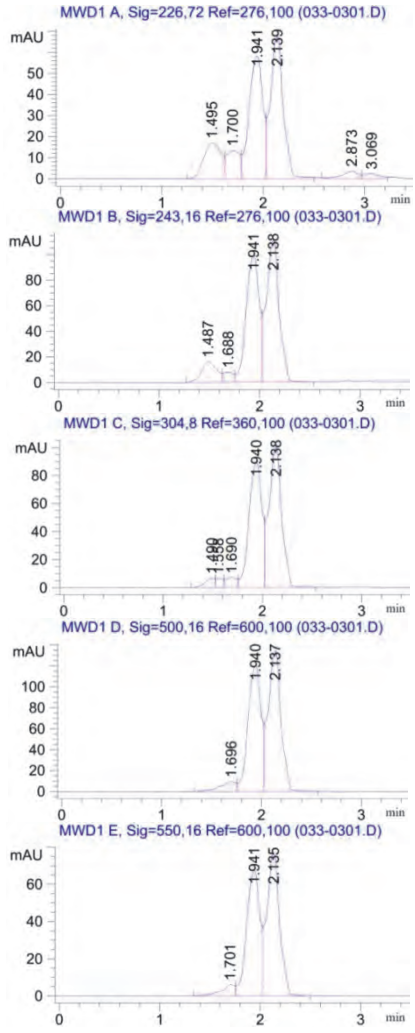

Fig. 2. HPLC-MWD chromatogram of Bemacid Rot dye.

As can be seen from Fig. 2, depending on the wavelength at which the chromatograms are recorded, the Bemacid Rot dye shows a different number of components. Thus at λ=226nm there are 5 components at λ=243nm, 4 components and λ=304nm, 5 components and at λ=500 and λ=550nm there are 3 components. Taking into account the peak integration area, it is 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%, respectively, of the total integration area. Furthermore, after biodegradation procedure of Bemacid ROT dye, HPLC analysis was carried out at 226nm, 243nm, 304nm, 500nm and 550nm wavelengths (Fig. 3).

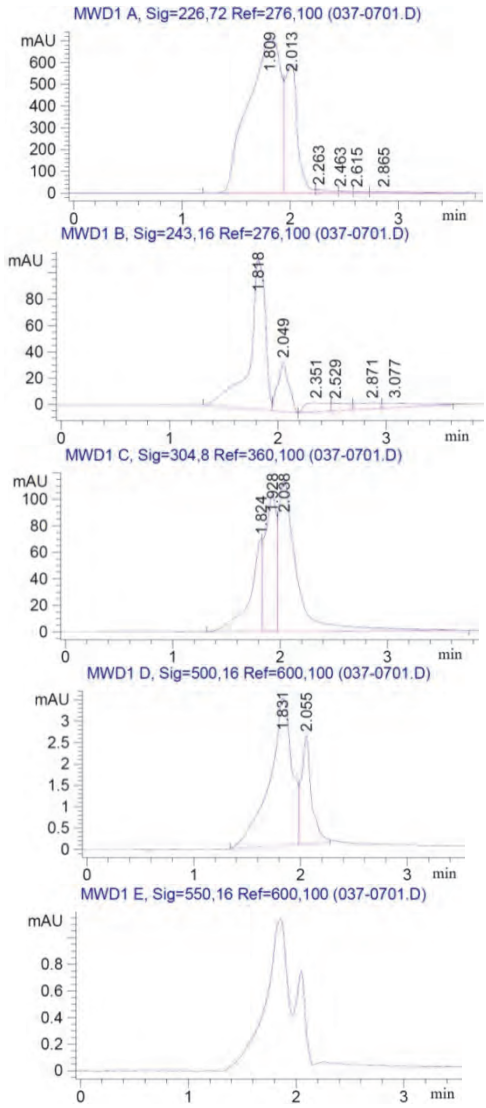

Fig. 3. HPLC-MWD chromatogram of biodegraded Bemacid Rot dye.

Although the proportion of the two major components and the retention times are slightly different from those of the standard dye, the chromatographic data demonstrate that at least some of the dye is still in the culture medium. The fact that at a wavelength of 234nm there is a multitude of components, with longer retention times than the dye, indicates the formation of biodegradation products less polar than the dye.

Figure 3 shows chromatograms of Bemacid Gelb dye at 200mg/L concentration at wavelengths of 228nm, 260nm, 277nm, 370nm and 436nm. The

absorption maxima of the dye, determined by UV-VIS, are at 370nm, the maximum peak, with 2 secondary peaks at 277nm and 436nm.

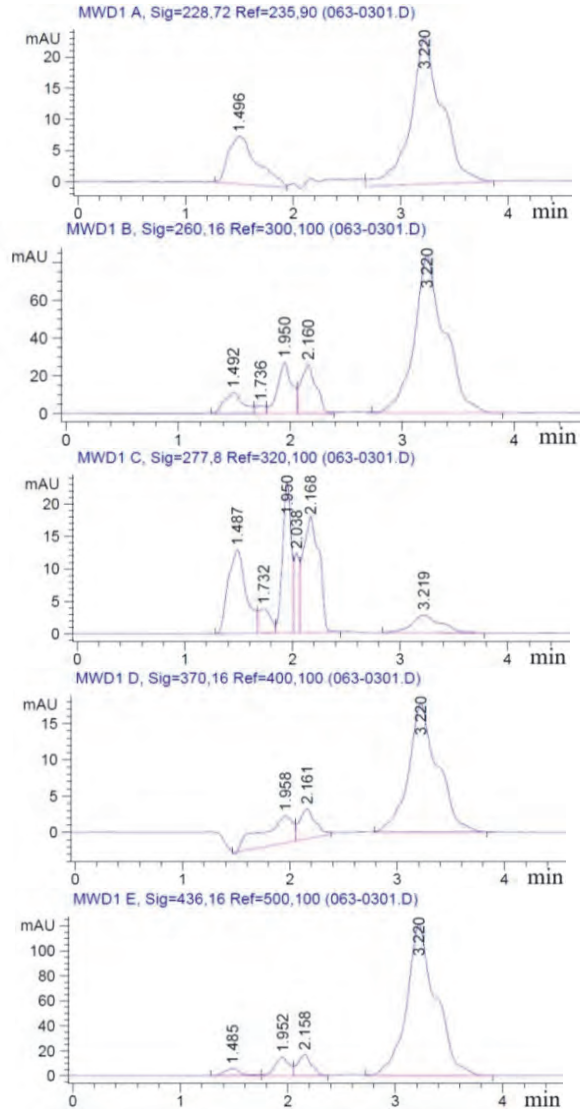

Fig. 3. HPLC-MWD chromatogram of Bemacid Gelb dye.

At concentration of 200 mg/L, the dye shows a major peak (71-86% area) eluted at about 3.2 minutes with other 4-5 minor peaks representing either isomers or synthesis impurities. On Bemacid Gelb dye, biodegradation compounds HPLC analysis, after *Aspergillus niger* activity, was carried out at 228nm, 260nm, 277nm, 370nm and 436nm wavelengths (Fig. 4).

Results indicate a significant reduction in the area of eluted compounds at 3.2 minutes, which may indicate a degradation of the dye. At the same time, it can be highlighted the presence of 4 peaks eluted at 260 nm, compared to 5 in the control sample, the presence of the same number of peaks at 277 nm, both in the treated and control samples, 2 peaks at 370 nm vs. 3 in the control sample and the decrease of the number of compounds eluted from 4 in the control sample to 3 in the case of 436 nm reading.

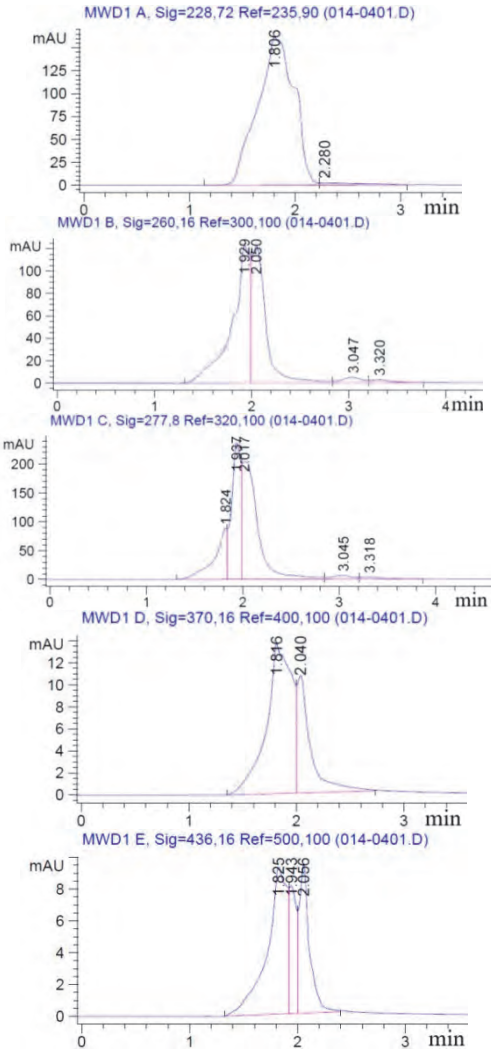

Fig. 4. HPLC-MWD chromatogram of biodegraded Bemacid Gelb dye.

On Bemacid Blau dye, HPLC analysis was run at 230nm, 260nm, 280nm, 590nm and 630nm wavelengths. The absorption maxima of the dye, determined by UV-VIS, are at 590nm, the maximum peak, with 2 secondary peaks at 629nm and 280nm (Fig. 5).

According to the obtained chromatograms, the dye exhibits a compound eluted at approximately 2.9 minutes, along with a secondary peak at about 3.2 minutes, which may represent a dye isomer.

At the same time, 4 other compounds are eluted from the column, between 1.5 and 2.3 minutes, which can be either dye isomers or synthetic impurities.

Biodegradation compounds chromatogram is presented in Fig. 6, obtained at wavelengths of 230nm, 260nm, 280nm, 590nm and 630nm.

Although 3 peaks are recorded at 590nm, their intensity is extremely small, and their quantification is not possible. Also, at the other wavelengths, the number of the resulting peaks is small, the absorption intensity of those of about 3 minutes

being reduced, which leads to the hypothesis of almost total degradation of the dye after biodegradative activity of *Aspergillus niger* strain.

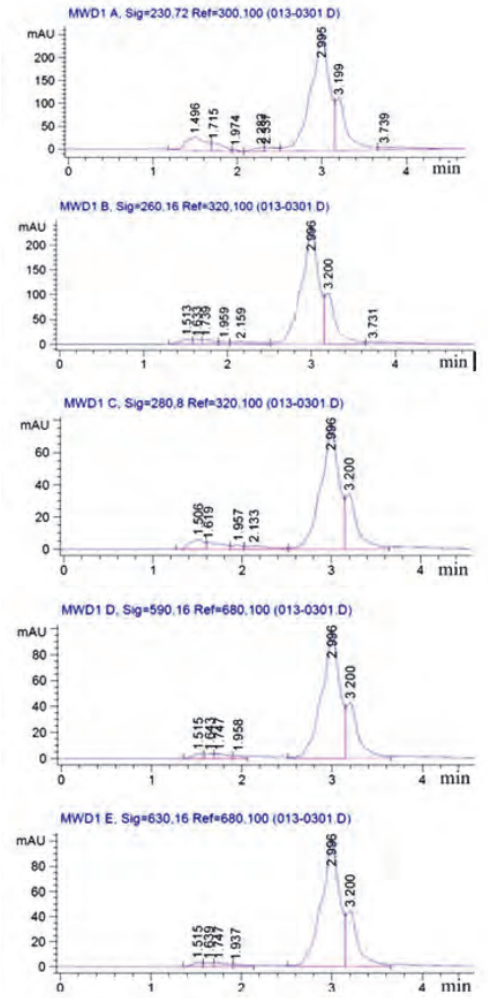

Fig. 5. HPLC-MWD chromatogram of Bemacid Blau dye **.**

5. CONCLUSIONS

HPLC analysis allowed the separation of the resulting compounds resulting from dyes biodegradation by *Aspergillus niger* strain, in relation to pure state dyes, as well as chromatographic signals of the nutritive media. Thus, for most of the dyes solutions analyzed, the decrease or even disappearance of the compounds eluted from the pure dyes solutions was observed, evidencing either their microorganism-mediated degradation or isomeric transformation. Analysis of the proportion of peaks and elution times, generally lower, reinforced the hypothesis of dyes degradation, with the formation of more polar compounds, which determined their elution faster on the column and the degradation of the functional groups and aromatic nuclei of the original dye, thus strengthening the great potential that filamentous fungi strains can have in bioremediation of heavily colored water effluents.

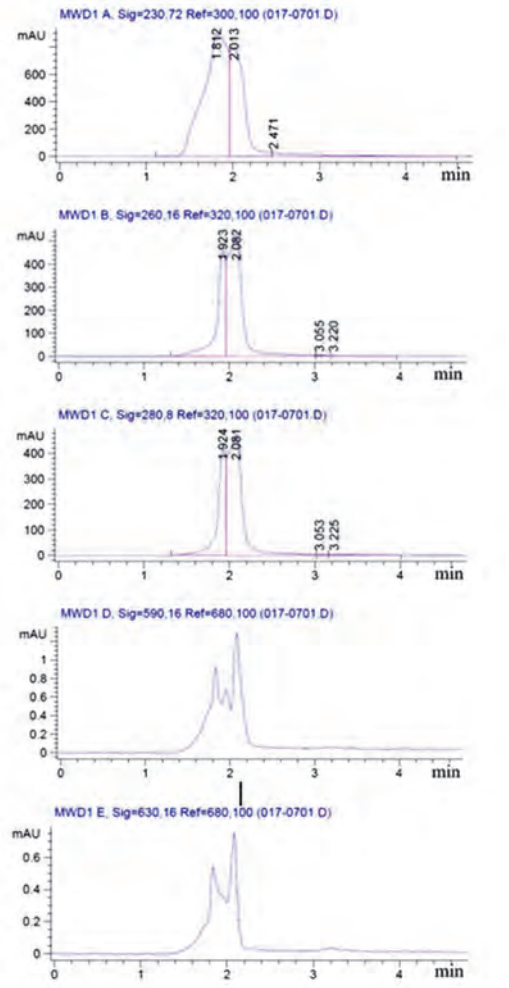

Fig. 6. HPLC-MWD chromatogram of biodegraded Bemacid Blau dye.

6. ACKNOWLEDGEMENTS

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