THE DECOLORIZATION MECHANISMS OF RESIDUAL EFFLUENTS FROM TEXTILE INDUSTRIES BY *CANDIDA INCOSNSPICUA$_{ICB-5}$*

CRĂIŢA – MARIA ROŞU[1], ŞTEFANIA SURDU[1], MARIUS MIHĂŞAN[1], ZENOVIA OLTEANU[2], LĂCRĂMIOARA OPRICĂ[2]

Key words: azoreductase activity, adsorbtion, azo – dyes, *Candida inconspicua$_{ICB-5}$*
Abstract: The testing of the yeast *Candida inconspicua* in the decolorization process of waste coloured water, resulted from the dye process of the textile fiber, revealed the presence of two possible mechanisms by azoic - dyes are removing from effluents, as follow: adsorbtion, correlated with a low azoreductase activity, in the case of Acid Blue 113 and biodegradation, as a result of reductive activity of azoreductase towards diazo = bonds, in the case of Basic Blue 41. The experiment revealed also, the dependence of enzymatic activity by temperature.

INTRODUCTION

The azo – dyes, which contains one or more azo (-N=N-) bonds, represents approximately 70% of textile dyes used in present. Being xenobiotic substance, their degradation in nature is very difficult. In this respect, yeasts represents a major advantage in the bioremediation process, because they can remove completely the aromatic amines resulted from the first step of the biodegradation. Also, they have the advantage of a rapid growth, like bacteria, and the resistance to the unfavorable condition of the environment, like fungi.

Usually, the removing mechanisms of the color dyes from industrial effluents, by the yeast cells, are the absorbtion or adsorbtion at the cell surface (Donmez, 2002 and Meehan, 2000). Martins and col. has already isolated a very efficient strain of Candida *zeylanoides*. The process can be associated with an azoreductase activity (Ramalho and col. 2002). The enzyme has a reductive activity of the azo = bonds. As a result, the aromatic amines are formed, which finally are completely mineralized by yeast. Therefore, the azoreductase producing microorganisms are important from a biotechnological point of view, especially in the first step of the bioremediation process.

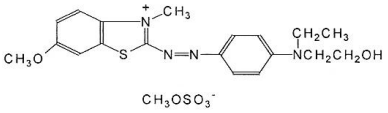
As referring to the azoreductase participation in the process, it has been documented the role of the redox mediators (ussualy quinonoid compounds) in the decolorization process, mediators that can facilitate the transfer of the reductive equivalents from the intracellular NAD(P)H to the azoic dyes, which are in the extracellular space. Also, has been reported that the presence of redox mediators in humic substances found in soils, enhance decolorization processes (Keck, 2002).

Azo – dye reduction process occurs, preferentially, in oxygen – limited or anoxic conditions (Nigam, 1996), when azo – dyes are acting as terminal acceptors of electrons, during the microbial respiration.

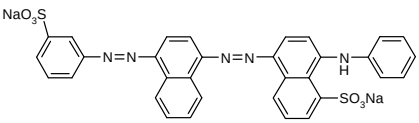
A very important aspect in the microorganism – textile dyes relationships it is represented by viability and physiology of a microorganism strain. In this respect, we have tested a posibble toxic effects of xenobiotic compounds on *Candida inconspicua* by comparing the growth curve in the presence and in the absence of the Acid Blue 113 and Basic Blue 41 and also, we have try to identify the decolorization mechanisms involved in the color removing process.

MATERIALS AND METHODS

Dyes, reagents and growth media: The textile azo – dyes Acid Blue 113 and Basic Blue 41, used in the experiments, were commercially available (Sigma). Also, media components were purchased from Merck and Difco and were, at least, analytical grade reagents.



Basic Blue 41



Acid Blue 113

In the biodegradation experiment of the dyes, 100 ml of mineral medium (MSM), previous tested (Rosu, 2007), with addition of yeast extract (0,2%) and glucose (0,5%), in 500 ml Erlenmeyer flasks, was used. The azo - dye initial concentration, in each flask, was 50 µl/ml. The glucose and dyes were filter sterilized. The final pH of the medium was 7,0.

Decolorization assay: During 24 hours of cultivation, every 6 hours, 10 ml of aliquots (yeast culture) were harvested and centrifuged (5 000 rpm) for 15 min and the supernatant was read at the maximum absorbance for each dye

(λ_{max} = 520 – Acid Blue 113) and (λ_{max} = 584 – Basic Blue 41). The decolorization efficiency of the *C. inconspicua*$_{ICB-5}$ was expressed, as follow: D % = (I-F)/I x 100, were: I = initial absorbance and F= absorbance of the decolorized medium.

For the identification of the already adapted yeast strain, API 32C (BioMérieux) was used, correlated with morphological characteristics. The growth of the yeast culture, in the presence of the textile dyes, was checked by harvesting, every 3 hours of 10 ml aliquots from the culture. Each sample was centrifuged at 5 000 rpm, 15 min, washed and dried 48 hours at 60^0C. The samples were weighted and the value expressed as g%.

Enzymatic assay: Azo - reductase activity was determined on pre – adapted and non-adapted yeast biomass. In the first experiment, adapted cells were harvested after 12 hours of cultivation in the presence of the selected dyes, in concentration of 50 µl/ml. In the second, the yeast cells were harvested after 6, 12 and 18 hours of cultivation, without dyes addition. In each test, the separated cells by centrifugation, were washed and resuspended in 0,05M phosphate buffer solution of pH = 4,0. The assay mixture (20 ml) contained: 10,4 Mm glucose, 1 mg dye, 0,1 mM AQS (anthraquinone – 2 – sulfonic acid) and approximately 150 mg wet biomass. The mixtures were incubated (in 50 ml flasks) for 2 hours at 30^0C and shaken at 100 rpm. During the assay, the dyes concentration decreased linearly with the time. The final absorbance was read at specific value for each dye and the azoreductase activity was expressed as micromoles degraded dye/hour/g wet biomass. As control, a mixture of reagents and biomass, without AQS, was used. Also, a calibration curve for each dye (stock solution -1 mg/ml dye) was constructed.

RESULTS AND DISCUSSIONS

The efficiency of the decolorization processes of the textile dyes is depending by the adaptability of the microorganisms to the xenobiotic compounds, by their biological activity and by chemical structure of the dyes.

In our experiments with *Candida inconspicua* $_{ICB\ -5}$ strain, already adapted to the textile dyes (Olteanu, 2008), presented a very good growth in the medium, in experimental conditions, in the presence of two kind of azo –dyes, with acidic (Acid Blue 113) and basic (Basic Blue 41) properties.

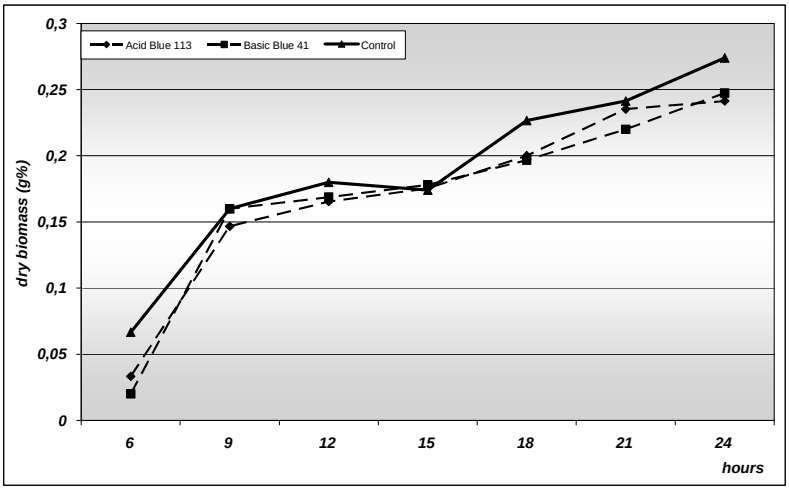


Fig. 1. Time – course of biomass acumulation of *Candida inconspicua*$_{ICB-5}$, in the presence of 50 µl/ml azo – dye (initial concentrations)

The *Candida inconspicua* $_{ICB-5}$ strain used has e very good growth, in the experimental conditions, in the presence of the selected azo – dyes (50 µg/ml). The dry weight of yeast biomass is significant between 9 – 15 hours of cultivation, at a similar level compared with control. The stationary phase of the growth is coresponding with the maximum rate of decolorization for both azo – dyes. After 12 hour of cultivation the decolorization rate was 78,5 % for Acid Blue 113 and 96,6% for Basic Blue 41 (Figure 2) at a biomass production of 0,165

g% dry weight in the presence of Acid Blue 113 and 0,168 g% dry weight in the presence of Basic Blue 41 (Figure 1). This fact is significant if we take in account the fact that, in many situations the decolorization process of industrial effluents is performed through adsorbtion of the dyes to the cell surface. After this period of time, the growth of the culture on ethanolic substrate resulted from the fermentation process of the glucose by the selected yeast has an insignificant effect on the decolorization process, except Acid Blue 113, were the value was 91,2% after 24 hours of cultivation. Taking in account this findings we suppose the the decolorization process is supported, additional, by a different mechanism of color removing from the medium, not only adsorbtion to the cell surface.

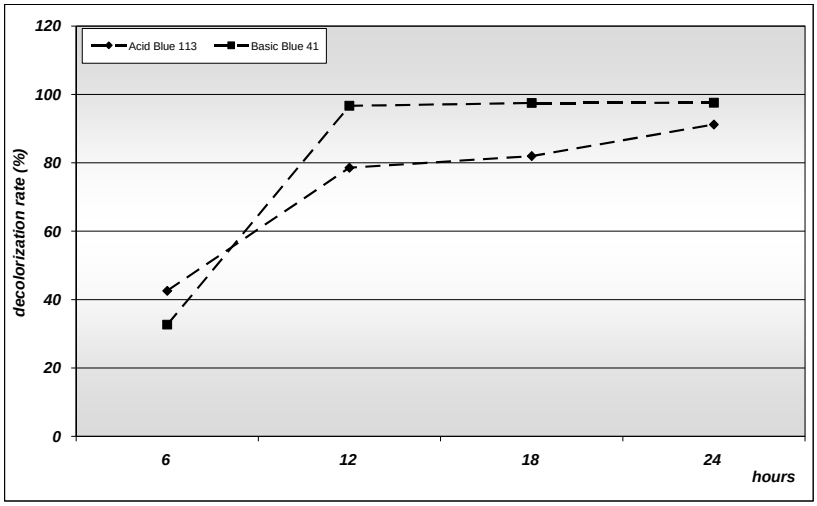


Fig.2. The decolorization rates (%) of the Acid Blue 113 and Basic Blue 41 by *Candida inconspicua*$_{ICB-5}$, in oxygen - limited conditions

For this reason, additional experiments were performed in order to asses the azo-reductase activity. Thus, the enzimatic activity was assesed with adapted and non-adapted yeast cells. The adapted yeast cells from the flasks with Acid Blue 113 and Basic Blue 41 dyes, additioned to the culture medium, were harvested after 12 hours of cultivation, centrifuged, washed with distiled water and resuspended in phosphate buffer 0,05M, pH = 4,0. Also, non – adapted yeast cells, from the dye free medium, were harvested in different growth stages (6, 12 and 18 hours of incubation) and prepared in the same way. The azoreductase activity was assesed using a redox mediator (AQS) (Ramalho, 2004).

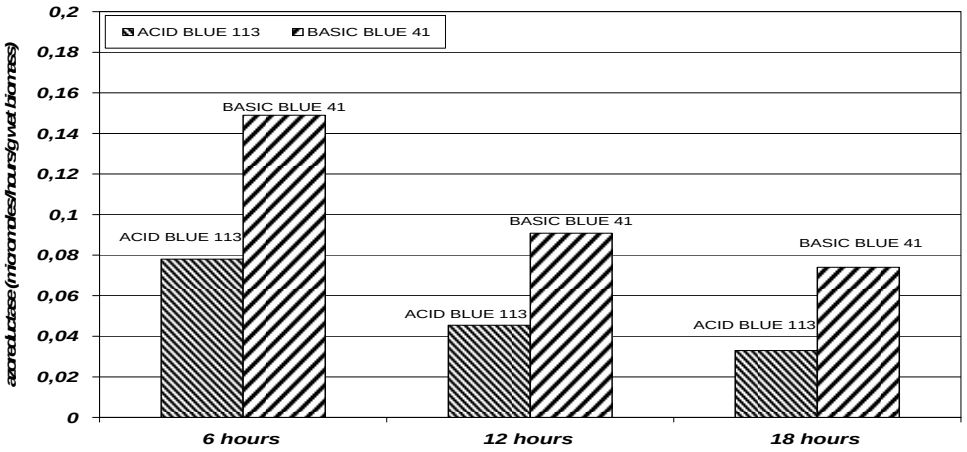


Fig.3. Azoreductase activity of *Candida inconspicua*$_{ICB-5}$ intact cells, non - adapted, harvested at different culture ages, at 30^0C, towards Acid Blue 113 and Basic Blue 41

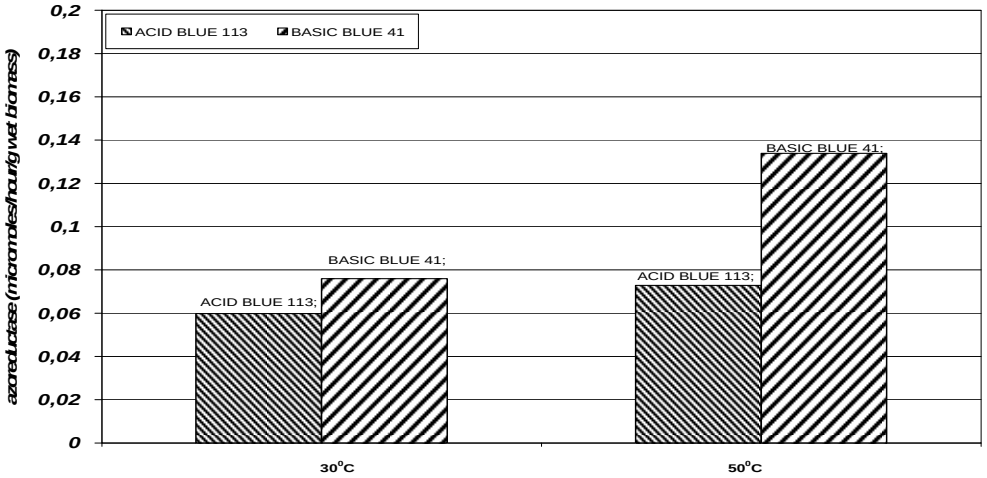


Fig. 4. Azoreductase activity of *Candida inconspicua*$_{ICB-5}$ intact cells, adapted, harvested after 12 hours of cultivation in the presence of the Acid Blue 113 and Basic Blue 41, assayed at 30^0C and 50^0C

As a result, the decolorization pattern of the two type of the yeast cells, used in the experiments, were similar. This fact allow us to postulate that azo- reductase is a constitutive enzyme in *Candida inconspicua* cells.

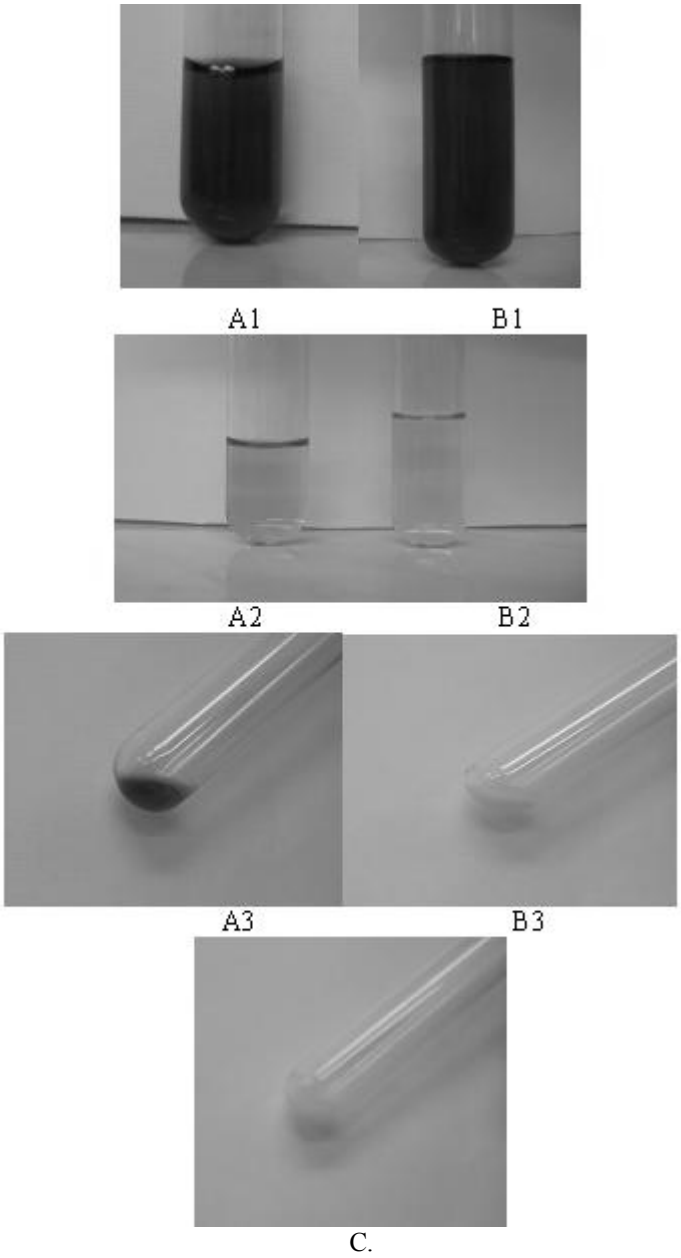


Fig.5. Biodegradation of Acid Blue 113 (A) and Basic Blue 41 (B) by *Candida inconspicua*$_{ICB\text{-}5}$ (A1and B1 – moment 0; A2, B2 and A3, B3 – free cells medium and the biomass aspect after 24 hour of cultivation in the presence of the dyes; C – control biomass)

The difference has been observed between the two textile dyes, used as enzyme substrate, depending on their chemical structure (Acid Blue 113 – 2 azo – bond and Blue Basic

41 – one azo – bond) and also, between the yeast cells ages (Fig.3 and 4). Thus, towards Basic Blue 41 dye the azoreductase activity (0,149 micromoles/hour/g wet biomass) was higher than towards Acid Blue 113 (0,078 micromoles/hour/ g wet biomass) in the case of 6 hours old non – adapted yeast cultures (in logarithmic growth phase).

Also, a very important fact noticed is the color of the yeast biomass at the and of the decolorization process of the Basic Blue 41 dye, which was white – creamy, specific for *Candida inconspicua* yeast (Fig.5). That means the bioremediation mechanisms, in this case, is based on azo - reductase activity. In the case of Acid Blue 113, the biomass color at the end of the process was blue, specific for the dye. The azo - reductase activity registered was not enough for the reducing of color intensity in the medium, the principal mechanism of the decolorization being the adsorbtion of the dye at the yeast cell surface.

CONCLUSIONS

The experiments demonstrated the existance of the two different decolorization mechanisms of the two azo – dyes, by *Candida inconspicua* cells, depending by their chemical structure: adsorbtion in the case of acidic dye - Acid Blue 113 (with 2 azo – bond) and a reductive reaction of azo – bonds, in the case of the basic dye Blue Basic 41 (with one azo – bond).

Also, in these experiments we have demonstrated the dependence of the azoreductase by temperature (increase with increasing temperature).

The decolorization rates (%), based on the two different mechanisms manifested by the yeast cells, achieved a maximum value after 12 hour of cultivation, for Basic Blue 41 (96,6%) and after 24 hours of cultivation for Acid Blue 113 (91,2%).

REFERENCES

Donmez, G., 2002. *Enzyme Microb.Technol*.30: 363 -366.

Meehan, C., Banat I.M., McMullan,G., Nigam, P., Smyth, F. and Marchant, R., 2000. *Environt.Int*. 26:75 -79.

Keck, A., Rau, J., Reemtsma, T., Matters, R., Stolz, A., and Klein, J., 2002. *Appl.Environ. Microbiol*., 68:4341 - 4349.

Martins, M.A., Cardoso, M.H., Queiroz, M.J., Ramalho, M.T. and Oliveira – Campos.A.M., 1999. *Chemosphere,* 38: 2455 -2460.

Olteanu, Z., Roşu, C.M., Mihăşan, M., Surdu, Şt., Oprică, L., 2008. *Analele Ştiinţifice ale Universitaţii „ Alexandru Ioan Cuza",* Secţiunea Genetică şi Biologie Moleculară, Tom IX: 41- 46.

Ramalho, P.A., Cardoso, H.M., Cavaco – Paulo A. and Ramalho, T.M., 2004. *Appl. Environ. Microbiol.* 70 (4): 2279 – 2288.

Roşu, C.M., Surdu, Şt., Olteanu, Z., Oprică, L., Mihăşan, M., 2007. *Book of Proceedings, 4th Symposium on Biosorbtion and Bioremediation*, Prague, August 26th – 30th: 94-97.

1 – Biological Research Institute, Iaşi

2 – University „ Alexandru Ioan Cuza", Iaşi, Faculty of Biology

* – email: craita2002@yahoo.com