PRELIMINARY CONSIDERATION UPON OXIDO-REDUCTIVE SYSTEM INVOLVED IN AEROBIC BIODEGRADATION OF SOME TEXTILE DYES

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Key words: yeast, biodegradation, textile dyes, oxidoreductive enzyme

Abstract: Coloured industrial effluents from dyeing industries represent major environmental problems because of their toxicity. In this paper, we present the analyses results of chemical and biochemical parameters involved in the decolourizations of some reactive (C.I. Reactive Red 120, C.I. Reactive Violet 5, C.I. Reactive Orange 16 and C.I. Reactive Red 243) and basic (C.I. Basic Blue 3, C.I. Basic Yellow 87, C.I. Acid Green 9) textile dyes (chemical oxygen consumption and catalase, peroxidase, dehydrogenase activities). The decolourization is realised by an adapted yeasts consortium, in aerobically conditions, isolated from activated sludge of a textile-water treatment plant. The results showed that both enzymes activity and chemical parameters are dependent on the chemical dye structure, concentrations, as well as cultivation conditions of yeasts consortium.

INTRODUCTION

The benefits generated by a rapid advance in textile dyes technologies are associated, many times, with environmental problems. The residual waters from textile industries or dyes industries are, some times, dangerous and toxic for plants and animals because of chemical structure of dyes and, also, of biogenes amines resulted from bacterial degradation of them. Many chemicals like detergents, surfactants, mineral oils, dyes and the degradation products of them are presents in residual waters. Their effects, like carcinogenetic potential, on living organisms, could be rapid or accumulated in time. Naturally, the textile dyes are degradated with difficulty. For this reason, their degradation could be obtain by physical, chemical or biological methods.

The identification of new microbial strains with biodegradation capacities upon textile dyes (Chen et al., 2007), the set up the conditions of cultivation of them, in order to reach the best degradation rate (Lucas et al., 2006), the elucidation of mechanisms involved in biodegradation processes (Baba Naoko et al, 2006), identification of the problems associated with their toxicity towards the environment (Bor -Yann Chen, 2002) and elaboration of biotechnologies for textile-water treatment plant (Mahdavi et al., 2001). , are the main subjects in scientific literature of the field.

In the last years, has been identified numerous microorganisms belonging to different systematic groups – bacteria, filamentous fungi, yeasts, actinomyces and algae, with the abillity to degrate the textile dyes.

The purpose of this study is to evaluate the viability of an yeast consortium, in some conditions of the biodegradation process, already established (Rosu et al., 2007), in the presence of different concentration of dyes with various chemical structure, by enzimatic assay at oxidoreductive level.

MATERIAL AND METHODS

The textile azo – dyes C.I. Reactive Violet 5 (Bezactiv Violet V – 3R; λ_{max}=560), C.I. Reactive Orange 16 (Bezactiv Orange V – 3R; λ_{max}=494), C.I. Basic Blue 3 (Astralon Blue BG; λ_{max}= 657), and C.I. Acid Green 9 (Bezanyl Grun F – 2B; λ_{max}= 646) used in the experiments were commercially available. The biodegradation experiments with the yeast consortium were conducted in mineral salt medium (MSM) of following composition (g.L $^{-1}$): Na_2HPO_4 – 3.6, $(NH_4)_2SO_4$ – 1.0, KH_2PO_4 – 1.0, $MgSO_4$ – 1.0, Fe (NH_4) citrate – 0.01, $CaCl_2.2H_2O$ – 0.1 and trace elements (10.0 ml solution per liter). The trace elements solution was prepared like stock solution, with following composition (mg.L^{-1}): $ZnSO_4.7H_2O$ – 10.0, $MgCl_2.4H_2O$ –3.0, $CoCl_2.6H_2O$ – 1.0, $NiCl_2.6H_2O$ – 2.0, Na_2MoO_4 .$2H_2O$ –3.0, H_3BO_3 – 30.0 and $CuCl_2.2H_2O$ – 1.0. The media components were purchased from Merck and Difco (yeast extract). All experiments were performed in 100 ml culture media, in 500 ml Erlenmeyer flasks, at 30^{0}C and 100rpm, in the presence of different concentration of dyes (C1=20 μg/ml; C2= 50 μg/ml and C3= 100 μg/ml).

The minimal medium was autoclaved at 121^{0}C for 15 min. The glucose, nitrogen source and dyes were filter sterilized.

The decolourization rate of the dyes by the yeast consortium was expressed, at 12 and 24 hours of cultivation, as follow (Khehra, 2005): decolourization (%) = (I – F)/I x 100, were I = initial absorbance and F = absorbance of decolorized medium.

The enzymatic activities had been estimated on integral media, also at 12 and respectively, 24 hours after the inoculation, in the presence of azo-dyes.

Determination of the dehydrogenases activity involve their capacity of transferring H_2 from endogenous substrates to the 2,3,5 triphenyltetrazolium chloride, which is reduced and transformed into triphenylformazane (Artenie, 1981)

Determination of catalase activity was achieved by titrimetric method (Artenie, 1981).

Determination of peroxidase activity assay was performed by o-dianisidine method. (Moller et al., 1966).

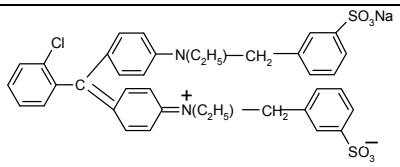
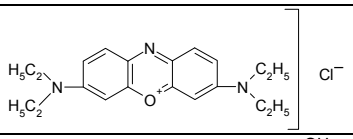
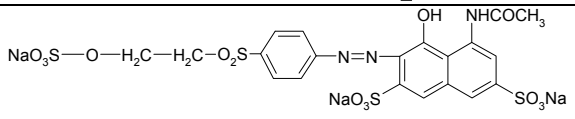
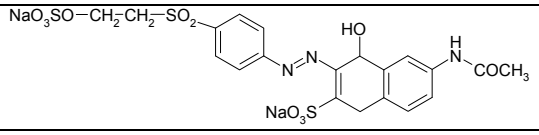
The CCOCr contents has been realised on culture supernatant (Mănescu, 1978).

RESULTS AND DISCUSSIONS

During the growing process of a yeast culture, could be distinguished three different stadia: the logarithmic growth, characterized by an intensification of a glycolytic way. The reductive step of glucose is followed by a modification at metabolic level, expressed by an increase in respiration process, known as diauxic shift, which is leading to stationary phase of the culture, were the metabolic rate is low and the yeast's multiplication is at down level. In this step, the antioxidant capacity of the microorganism is increased and the culture can survive a longer period of time without nutrients add (Longo, 1999). The azo – dyes added to the culture, influenced the growing process of the culture and, also, the metabolic behaviour.

The decoloration rate (%) of the dyes is influenced by the their chemical structure and properties, and, also, by the concentrations added to the culture media. (Table I).

Table I – The chemical structure and the decoloration rate (%) of some textile dyes, according with time and concentration, in the presence of an yeast consortium

Chemical structure	Name	Concentration µg/ml	Decoloration rate (%)	
			12 hours	24 hours
	C.I. Acid Green 9 (BEZANYL GRUN F-2B)	20	0	11.16
		50	25.00	18.81
		100	52.31	17.39
	C.I. BASIC BLUE 3 (ASTRAZON BLUE BG)	20	24.42	14.42
		50	29.36	0.23
		100	29.17	4.82
	C.I. REACTIVE VIOLET 5 (BEZACTIV VIOLET V-5R)	20	79.38	89.30
		50	59.73	78.05
		100	48.80	70.98
	C.I.REACTIVE ORANGE 16 (BEZACTIV ORANGE V – 3R)	20	60.74	99.10
		50	56.35	99.49
		100	17.74	53.09

The reactive dyes Orange 16 and Violet 5 are degraded faster than Basic Blue 3 or Acid Green 9. The continue growth of the degradation rate (%) till 24 hour of cultivation, in the case of the reactive dyes, suggests that the degradation is continue all this period of time. The decrease of this rate, between 12 - 24 hours of cultivation, in the case of basic and acidic dyes,

added to the culture medium, is a result of liberation of the dyes from yeast cells, after an initial adsorption of them. For these dyes, the removing process from the medium is increased in the first 12 hours of incubation only, at high concentrations.

Increasing of the oxidoreductive enzyme activity, in environmental stress condition, represents an efficient way of protection of cells towards reactive oxygen species (ROS), like: super –oxide radical (O_2^{2-}), the hydrogen peroxide (H_20_2) and the hydroxyl radical (OH^-), reactive species which are the result of oxidoreductive reactions, involved in cell metabolism. The research shows that mitochondrial respiration is a major source of ROS in stressed cells, produced even at low level of aerobiosis, when the cell can use the enzyme with antioxidant properties to defend themselves (Martins et al., 2005). The catalase is one of the most efficient enzyme which react with H_2O_2 to form H_2O and molecular oxygen. The peroxidases remove the hydroperoxides (ROOH and H_2O_2) by coupling their reduction to water with the oxidation of a specific substrate for which they are dependents (Matés, 2000).

The evaluation of actual and potential dehydrogenase activity let us to appreciate the general level of the consortium metabolism, the oxidoreductive enzyme being involved in various metabolic ways. Towards controls, the obtained results suggest the fact that adding the textile dyes in culture medium influenced the enzymatic activity of the culture more by their chemical structure than the concentrations. (Fig. 1).

The low level of dehydrogenase activity in the yeast culture with Acid Green 9 dye, suggest also, that the growing process and the metabolic processes intensity are considerable reduced compared with control culture, in the first 12 hours of cultivation. The next 12 hours of cultivation is accompanied by a diminution of dehydrogenase activity, as low as in control variants.

In the experimental conditions, the oxidoreductive enzyme activities, with antioxidant properties, are different according with different level of ROS, in a constant state of aeration.

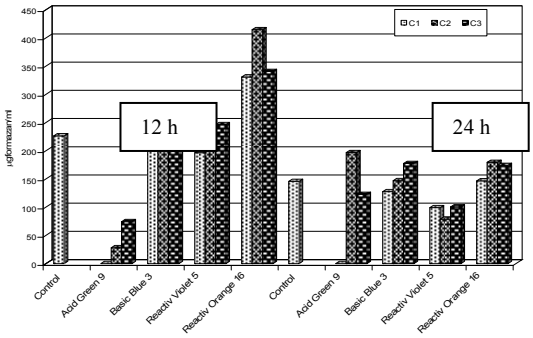


Fig. 1 – Dehidrogenase activity at 12 and 24 hours of cultivation of yeast consortium in the presence of different concentration of textile dyes (C1=20 μg/ml; C2= 50 μg/ml and C3= 100 μg/ml)

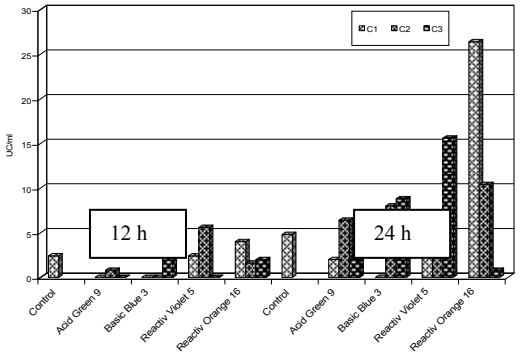


Fig. 2 - Catalase activity at 12 and 24 hours of cultivation of yeast consortium in the presence of different concentration of
textile dyes (C1=20 μg/ml; C2= 50 μg/ml and C3= 100 μg/ml)

In the first 12 hours of cultivation the catalase level is low, but with a significant increasing in the next period of cultivation (Fig.2). The reactions at cell level are different, according with dye concentration. The most active culture are in the presence of reactive dyes, with highest level of catalase activity, associated with higher level of decoloration rate.

The peroxidase activity level is reduced towards control, this could be assayed in the first 12 hours of cultivation (Fig.3). At lowest concentration of dyes, the activity level is the same as in control culture. At 100μg/ml dye, the peroxidazic activity of the culture is diminished toward controls, in the presence of Basic Blue 3, Reactiv Violet 5 and Orange 16. Also, Acid Green 9 reduce significant the enzymatic activity.

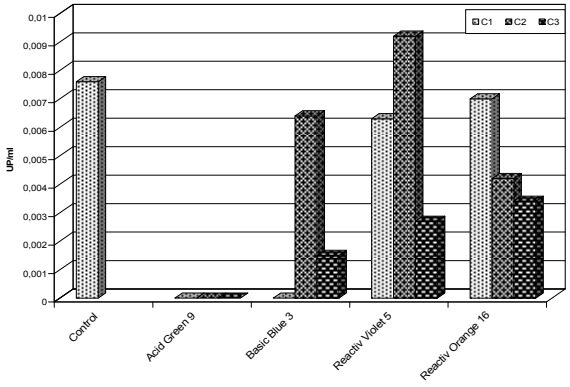


Fig. 3 Peroxidase activity at 12 and 24 hours of cultivation of yeast consortium in the presence of different concentration of textile dyes (C1=20 μg/ml; C2= 50 μg/ml and C3= 100 μg/ml)

The inverse ratio between catalase and peroxidase activity could indicate the cultures capacity to adapt their own enzymatic system to the specific cultivation parameters, in principal to the dyes concentration, the essential role being played by catalase.

The CCOCr evaluation, in the case of 50μg/ml dye added to the medium, in a constant level of oxigenation of the culture, shows a constant chemical consume of oxygen in all variants, in the first 12 hours of cultivation, except in the case of Reactiv Orange 16. The registred value

for CCOCr decreased after 24 hours of cultivation, except the variant with Basic Blue 3 (Fig. 4). This behaviour, allow us to consider that organic or anorganic compounds, which can be oxidated in cold condition, are quantitatively diminished and decrease the level of pollution in these biodegrated waters.

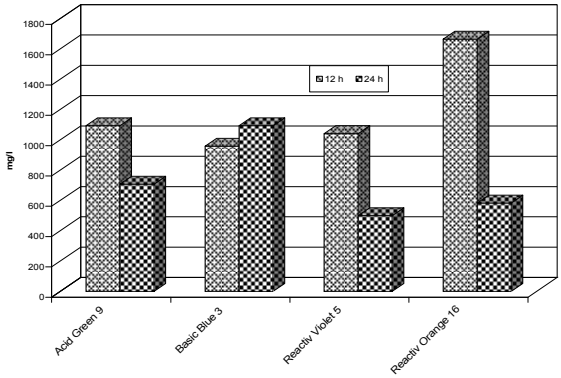


Fig. 4 – CCOCr at 12 and 24 hours of cultivation of yeast consortium in the presence of different concentration of textile dyes (C1=20 μg/ml; C2= 50 μg/ml and C3= 100 μg/ml)

CONCLUSIONS

The yeast consortium used in this experiment has the capacity to degrade the azo – dyes in different ways, according with their chemical structure and the concentration added in the cultivation media.

The consortium has the ability to respond differently to oxidative stress, generated by the dyes, the first role being played by catalase, in order for the consortium to survive and to diminish the negative effects from the environment. During the biodegradation process we can notice a tendency of equilibration between catalase and peroxidase activity, an important behaviour in the removing ROS process.

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