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Characterization of the azo reductase activity in a novel ascomycete yeast strain

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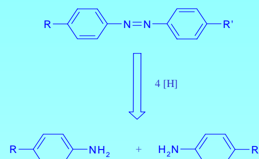
INTRODUCTION

A considerable volume of work has been developed over the last two decades, aiming at the application of microorganisms as bioremediation agents for the treatment of wastewater containing textile dyes. These contaminants contribute a minor fraction to the usually high load of dissolved organic matter in textile effluents but they are highly visible, and must be removed in order to comply with the regulations concerning effluent discharge.
The few reports on bioremediation of coloured effluents by yeasts usually mention biosorption as the major cause for decolourisation. Our research group, however, has isolated a strain of *Candida zeylanoides*, which efficiently decolourises several azo dyes [1]. Further work demonstrated that a reductive cleavage of the azo bond was involved in this process, and described some characteristics of the corresponding azoreductase activity [2]. In this work we examined the decolourising activity of a novel yeast strain, UM41. This species is even more efficient than *C. zeylanoides* in decolourising the previously tested dyes. It was therefore decided to investigate, in more detail, the effects of several parameters on the azoreductase activity of UM41.

Reduction mechanism



OBJECTIVES OF THE WORK

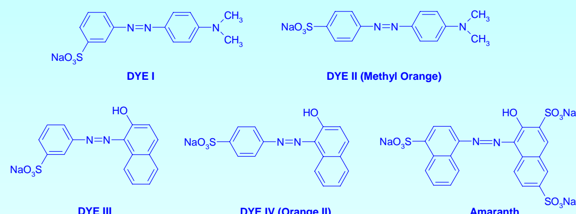
This work intends to characterize the azoreductase activity in UM41, and its decolourising performance under several conditions. The evidence obtained from this study is expected to provide a sound basis for the development of a biotreatment process for azo dye-containing wastewaters.

MATERIALS AND METHODS

Microorganism

Strain UM41

Dyes



Decolourisation in liquid media

- Normal Decolourisation Medium (**NDM**) (% w/v): $(NH_4)_2SO_4$ (0.25), yeast extract (0.25), KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.05), $CaCl_2$ $2H_2O$ (0.013) and glucose (2);
- 0.2 mmol.L^{-1} of dye.

Activity assays

- Cells grown for 14 hours;
- Ressuspended in 0.05mM phosphate buffer solution;
- Assay mixture (in a total volume of 20 mL): 0.019mM of glucose, 0.047mM of the dye and 0.1mM of AQS;
- The assays where performed in 50 mL cotton-plugged Erlenmeyer flasks, shaken at 120 rpm and 26ºC for 2 hours.

RESULTS

Decolourisation experiments

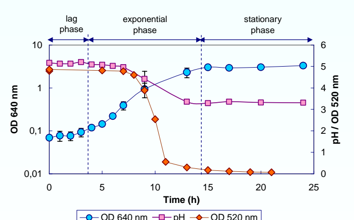


Figure 1: Typical growth and decolourisation curve of UM41 on NDM in the presence of dye II.

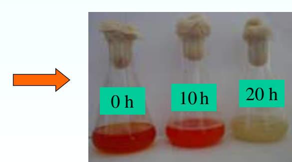


Figure 2: Aspect of the flasks after 0, 10 and 20 hours of growth.

RESULTS

Constitutive azoreductase activity

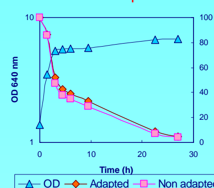


Figure 3: Effect on the decolourisation process of the pre-adaptation of the cells to the dye.

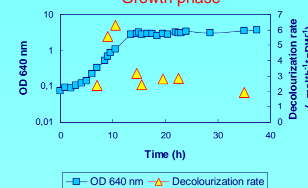


Figure 4: Effect of the growth phase on the decolourisation rate.

Parameters affecting the enzyme activity

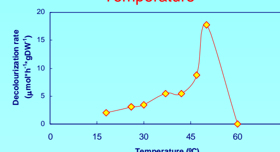


Figure 5: Effect of the temperature on the decolourisation rate.

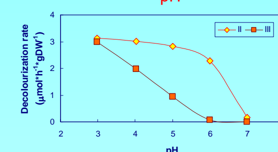


Figure 6: Effect of the pH on the decolourisation rate.

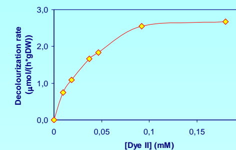


Figure 7: Effect of the dye concentration on the decolourisation rate. The kinetic parameters are: $v_{máx}$ = 3.2 μmol.h^{-1}.g^{-1} and K_M = 0.034 mM.

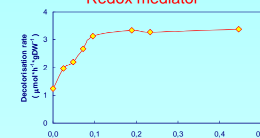


Figure 8: Effect of the redox mediator on the decolourisation rate.

Maximum capacity of decolourization

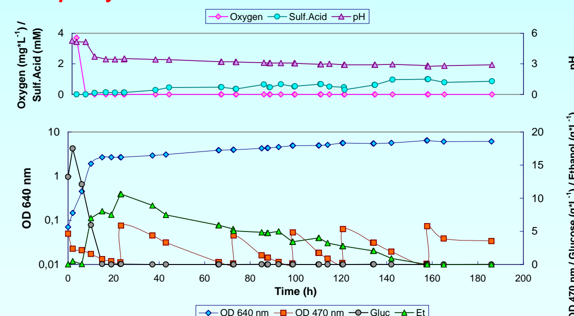


Figure 9: Repeated cycles of decolourisation by the same batch of UM41 cells. Sulfanilic acid, one of the reduction products of dye II, was detected in the supernatant medium.

CONCLUSIONS

- The specific growth rates observed in the presence of dyes and in control cultures were in the range 0.29-0.36 h^{-1}, suggesting that none of the dyes significantly inhibited the microorganism growth.
- Typically, most of the tested dyes are removed after 15 h incubation (80-96%), and total decolourisation is observed at 24 h, without any visible dye adsorption to yeast cells.
- The azoreductase activity seems to be constitutive in the yeast cells.
- Maximum activity was observed for cells collected in the late exponential phase.
- The bell shaped form of the curve of temperature supports the participation of a enzymatic activity on the dye reduction.
- The dye structure influences the rate of decolourisation.
- As with bacteria, it seems that the primary azo dye reductant is an intracellular species which transfers reducing equivalents to the extracellular dye through a redox shuttle.
- The results confirm that dye decolourisation by this yeast strain is due to azo bound reduction.
- It is needed an external carbon source for the decolourisation process.
- Azo dye reduction seems to occur under oxygen-limiting conditions, even in the respiratory phase.

➡ The results of this work show the potential of the yeast strain, UM41, for bioremediation of acidic effluents from textile industry.

FUTURE RESEARCH

Further work is needed especially about the effects of other components rather then dyes on the performance of the yeast.

REFERENCES

[1] **Martins M.A., Cardoso M.H., Queiroz M.J., Ramalho M.T., Oliveira-Campos A.M.** 1999. Biodegradation of azo dyes by the yeast *Candida zeylanoides* in batch aerated cultures. Chemosphere. **38**:2455-2460.
[2] **Ramalho P.A., Scholze H., Cardoso M.H., Ramalho M.T., Oliveira-Campos A.M.** Reductive aerobic decolorization of azo dyes by C. zeylanoides. Enzyme Microb.Technol. (in press).