STUDIES OF DECOLORIZATION OF AZO DYES BY ASCOMYCETE YEASTS

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INTRODUCTION

Azo dyes are the most widely used colouring materials in textile industries and its biodegradability is, therefore, an important issue in the biological treatment of waste waters containing dyes. However, existing treatment processes are not totally effective in removing colour of textile dye waste-water since those substances are tipically resistant to oxidative degradation.

Most biodegradation studies on azo dyes involve bacterial species, and anaerobic or microaerophylic conditions are usually refered to as being favourable to a reductive step producing colorless amines. However our group has succeeded in isolating a number of ascomycete yeast species, from dye-contaminated environments, which revealed to be highly efective in the colour removal of azo dyes, by a reductive mechanism, in aerated culture media. Three of them *(UM2, UM41* and *UM45*) were used in the present work. We have investigated, for several model azo dyes (i) the time course of the the decolorization process, monitoring simultaneously biomass and pH, (ii) the effect of the reduction of yeast extract and glucose concentrations on the decolorization process, (iii) the effect of pre-adaptation of the yeast strains to the dyes on decolorization times and (iv) assimilation of metanilic or sulfanilic acids, which are formed upon decolorization by the tested microorganisms. The relative colour removal efficiencies of the three strains were also tested against a number of commercial reactive textile dyes.

MATERIALS AND METHODS

Microorganisms The ascomycete yeast strains *UM2, UM41 and UM45* were isolated from dye-contaminated soil and water samples.

Culture media. The normal decolorization medium (NDM) contained mineral the usual mineral salts, yeast extract and 2% glucose. The improved decolorization medium (IDM) contained mineral salts as above, 1% glucose and was supplemented with vitamins and oligoelements. In either case, excepted when stated otherwise, media were made 0.2mM in dye. Assimilation of dye-reduction products was tested in minimal media (YNB and YCB) with 5mM substrate.

Dyes. The structures of the model azo dyes and the commercial names of the reactive dyes used in this work are represented in figures 1 and 5, respectively

Experimental conditions. Decolorization and assimilation experiments were performed in 250 ml conical flasks containing 100 ml of the appropriate medium. The flasks were incubated (120 rpm, 25ºC) in an orbital incubator shaker. Inocula were cell suspensions prepared from fresh slants except when the effect of pre-adaptation of the microorganism to the dyes was being tested. In these experiments the cells grown overnight in 100 ml NDM, with or without dye, were used as inocula of 100 ml of dye containing media.

HPLC analyses. The analysis of dye reduction products in the culture media was performed by high performance liquid chromatography (HPLC) using a Lichrocart 250-4 cartrige packed with Lichrospher RP-18, 5mm, eluted with methanol/water (30:70 v/v), containing 5mM tetrabutylammonium phosphate (TBAP).

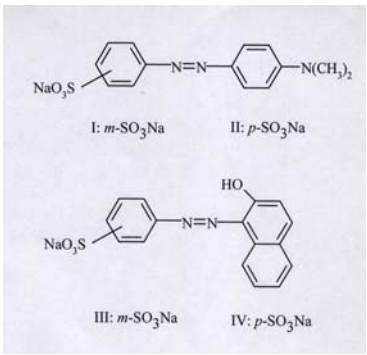

Figure 1. Structures of model azo dyes.

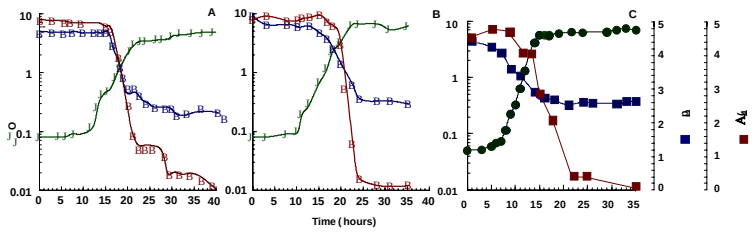

Figure 2. Typical plots of dye decolorization (B), cell growth (J) and pH (B) *vs.* time (hours) for dye I, obtained in the presence of *UM2, UM41* and *UM45*. Initial dye concentration=0.2mM.

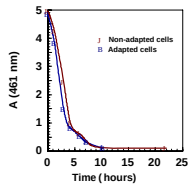

Figure 3. Comparison of the decolorization progress for dye I by adapted and non-adapted cells of *UM 2*. Initial dye concentration=0.2mM.

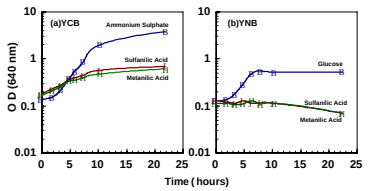

Figure 4. Growth of *UM2* in minimal media. (a) YCB, with 5mM metanilic or sulfanilic acid as nitrogen source, with a positive control (2.5mM ammonium sulphate). (b) YNB, with 5mM metanilic or sulfanilic acid as carbon and energy source, with a positive control (5mM glucose).

RESULTS AND DISCUSSION

As displayed in figure 2 for dye I, decolorization tipically occurs between the mid and the late exponential growth phase. The specific growth rates are not affected by the dyes (results not shown) ranging, in either situation, between 0.25 and 0.35 h^{-1}.

The pH decrease is related, as expected, with glucose consumption and seems to play a role in the decolorization process. In fact, in experiments performed at a fixed pH of 5, decolorization did not occur (results not shown). Similar results were obtained with the other model dyes. Some differences were detected, however, in the decolorization efficiencies of the three strains. With *UM 41* in NDM, total colour removal of dyes I-IV was observed in 25 h, whereas with *UM 2*, in the same medium, colour desappearence occurred in 40-60 h. As for *UM 45*, in IDM, the decolorizarion times were similar to those observed with *UM 41* in NDM.

Decolorization times are virtually unaffected by the use of a pre-adapted inoculum, as observed with all the model dyes and yeast strains. Figure 3 illustrates the results corresponding to dye I and strain *UM 2*. The only consistent differences detected in these experiments relate to decolorization times, which depend on dye structure (results not shown). The enzyme activity responsible by azo bond reduction is, therefore, constitutive. The structural effects are probably related to the dye redox potential but the elucidation of this factor will require further investigation.

The sulphonic moieties of dyes I and III generate metanilic acid upon reduction, whereas the corresponding moieties of dyes II and IV produce sulfanilic acid. These two compounds were therefore tested as possible carbon and energy souces, in YNB, or as nitrogen sources, in YCB. Results shown in figure 4, for *UM2*, show that metanilic acid and sulfanilic acid can be used as nitrogen sources but not as carbon and energy sources. Similar results were obtained with the other strains.

The potential of the these yeast strains in the decolorization of commercial reactive textile dyes is well documented in figure 5. Seven of the nine dyes where fully decolorized in 16-18h by the three strains.

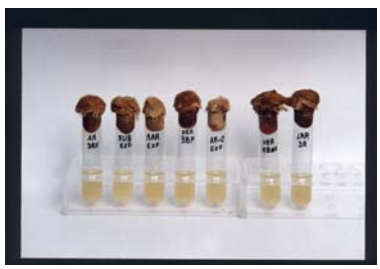

Figure 5. Decolorization tests of commercial textile dyes by *UM45* in IDM. (a) Initial aspect; (b) after 18-24h incubation

Figure 6. Partial decolorization tests of commercial textile dyes by *UM45* in IDM after 24h incubation.

CONCLUSIONS

An azoreductase activity was detected in several yeast species isolated from dye-contaminated soil and water samples. Such an activity is constitutive and comparatively inespecific. It seems, however, that decolorization rates are affected, to some extent, by the dye structure. The decolorization efficiencies of the tested microorganisms compare favorably with those of bacteria and white-rot fungi, which are also being investigated for possible application in textile waste-water treatment.