REDUCTIVE BIOLOGICAL TREATMENT OF TEXTILE EFFLUENTS

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PORTUGAL
• Collection of contaminated soil from a treatment plant that receives large amounts of coloured water
• Growth in rich medium to select microorganisms
• Isolation of yeasts in selective medium
• Growth of yeasts in YEPD-agar medium with a model azo dye to select strains with decolourising capability
• Identification of the yeasts with better decolourising activity: Candida zeylanoides (UM2) and Issatchenka occidentalis (UM41)

Study the decolourisation process by YEASTS
Selection of model azo dyes

Dye I

Dye II (Methyl Orange)

Dye III

Dye IV (Orange II)

Amaranth

Ramalho et al. 2002 **Improved conditions for the aerobic reductive decolourisation of azo dyes by *Candida zeylanoides* Enz Microb Technol 31: 848-854
Decolourisation in batch:

Conditions:
- 0.2 mM dye
- NDM with 2% glucose
- 120 rpm
- 26°C

Ramalho et al. 2002 Improved conditions for the aerobic reductive decolourisation of azo dyes by Candida zeylanoides Enz Microb Technol 31: 848-854
The decolourisation process by yeasts

Decolourisation in batch - results:

- The presence of dyes in the growth medium does not affect the specific growth rates;
- Yeasts are able to reduce azo dyes to colourless amines;
- This activity is constitutive;
- Depends on dye structure.

Rimalho et al. 2002 Improved conditions for the aerobic reductive decolourisation of azo dyes by *Candida zeylanoides* Enz Microb Technol 31: 848-854
Decolourisation process by yeasts

Decolourisation in batch - results:

• Dye degradation is connected to growth phase;
• The glucose is fermented and the resulting ethanol is respired;
• O₂ in the incubation conditions is limited;
• Under anoxic or aeration conditions there is no decolourisation;
• Neither dyes nor resulting amines are toxic to yeasts;
• Yeasts can use the produced amines as carbon and nitrogen sources.

Rimalho et al. 2004 Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain
Appl Environ Microbiol 70: 2279-2288
The decolourisation process by yeasts

Activity assays - results:

Effect of dye concentration

Effect of growth phase

\[ r_{\text{dye max}} = 3.2 \, \mu\text{mol}\,h^{-1}\,g^{-1} \]

\[ k_M = 0.034 \, \text{mM} \]

Ramalho et al. 2004 *Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain* Appl Environ Microbiol 70: 2279-2288
Activity assays - results:

**Effect of temperature**

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**Effect of pH**

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Ramalho et al. 2004 *Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain*  
Appl Environ Microbiol 70: 2279-2288
**The decolourisation process by yeasts**

**Maximum capacity of decolourisation:** 0.5 mM dye/ g glucose

Ramalho et al. 2004 *Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain*  
Appl Environ Microbiol 70: 2279-2288
The decolourisation process by yeasts

Results:

- There is a need of an external carbon source;
- The activity is only detected in intact cells;
- Can use alternative carbon sources like acetate and ethanol.

The enzyme system

From literature -> yeasts have a plasma membrane redox system to reduce iron(III) to iron (II) (solubilization of iron) – **THE FERRIC REDUCTASE SYSTEM**

Could this system be responsible for the azo reductase activity in yeast cells?

Selection of laboratorial *Saccharomyces cerevisiae* strain with the ability to degrade azo dyes

I. Measurement of both ferric and azo reductase activities along growth and decolourization:

Ferric and azo reductases have **parallel activity curves** with maxima in the late exponential growth phase.

Ramalho et al. 2004 *Evidence for two distinct electron donor sites on azo reductase activity in the yeast Saccharomyces cerevisiae* Submitted to J Biol Chem
Strategies to prove the new functionality of ferric reductase

II. Addition of an known inhibitor of ferric reductase (IRON) to the decolourisation medium:

The addition of iron to the medium inhibits ferric reductase at both transcriptional and post-transcriptional levels (Lesuisse et al. 1996) and delays decolorization

Ramilho et al. 2004 Evidence for two distinct electron donor sites on azo reductase activity in the yeast *Saccharomyces cerevisiae* Submitted to J Biol Chem
III. Deletion of the genes *FRE1* and *FRE2* in the strain: effect on azo and ferric reductase activities

The deletion of *FRE1* gene removes the decolorizing activity; *FRE2* removal does not affect the decolorizing ability, in our conditions.

Ramaelho et al. 2004 *Evidence for two distinct electron donor sites on azo reductase activity in the yeast Saccharomyces cerevisiae* Submitted to J Biol Chem
Strategies to prove the new functionality of ferric reductase

III. Deletion of the genes FRE1 and FRE2 in the strain: effect on decolourisation activity

Fre1p is responsible for the major part of the azo reductase activity of intact yeast cells, but there is an alternative reductase in yeast cells.

Ramaelho et al. 2004 Evidence for two distinct electron donor sites on azo reductase activity in the yeast Saccharomyces cerevisiae Submitted to J Biol Chem
Conclusions

- Yeasts are able to reductively decolourize azo dyes in acid effluents
- It is needed an external carbon source, that can be acetate, ethanol or glucose
- Oxygen is needed in limited conditions
- The amines produced are used as carbon and nitrogen sources – the complete mineralization can be achieved
- The enzyme system responsible for the major part of this activity is the plasma membrane ferric reductase
Future perspectives

• Production of a bioreactor to study the effect on the decolourisation process of salts, pH and temperature and test real effluents

• Genetic manipulation of the *Saccharomyces cerevisiae* strain to overexpress the enzyme system
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