Study of the effect of redox mediators on the decolorization of azo dyes by ascomycete yeasts

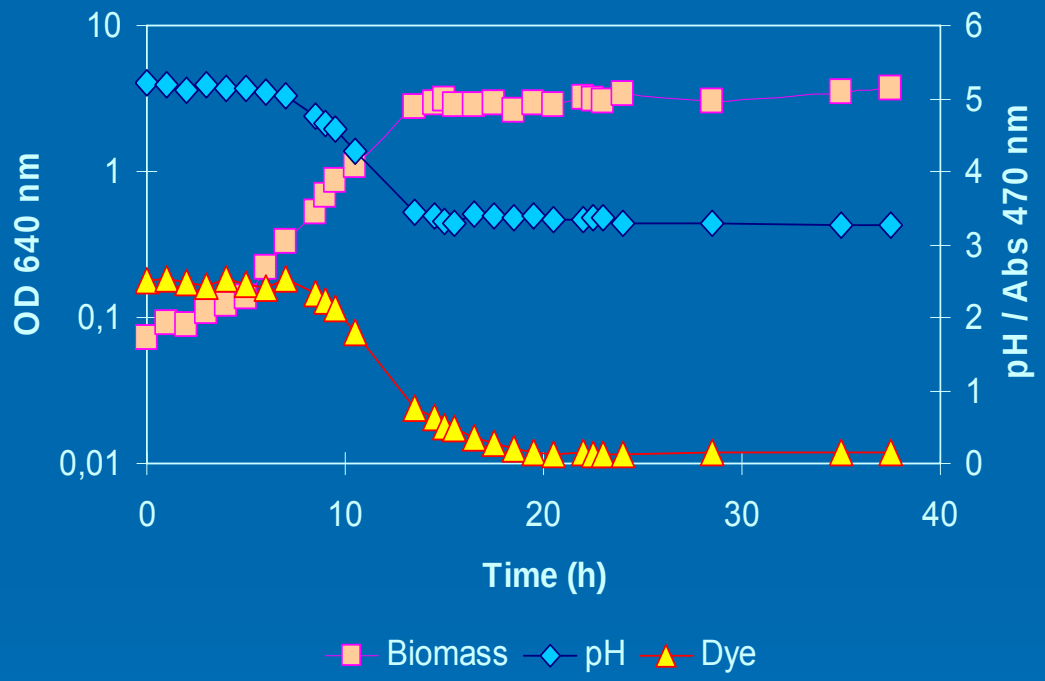
Biology Dept., Chemistry Dept. and Textile Engeneering Dept., University of Minho

P.A.Ramalho, M.H.Cardoso, R. Millward, A.Cavaco-Paulo, M.T.Ramalho

Introduction

- Some yeasts can reductively cleave azo bonds in dyes, producing colourless amines.
- Reduction is growth- and pH-dependent.
- The available evidence suggests the involvement of an enzymatic activity in azoreduction.
- Azoreductase activity is constitutive.

The decolorization process by UM41

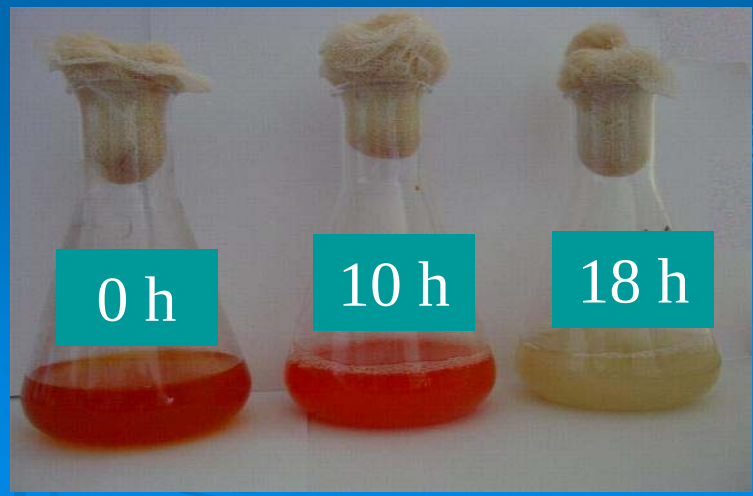


The strain:

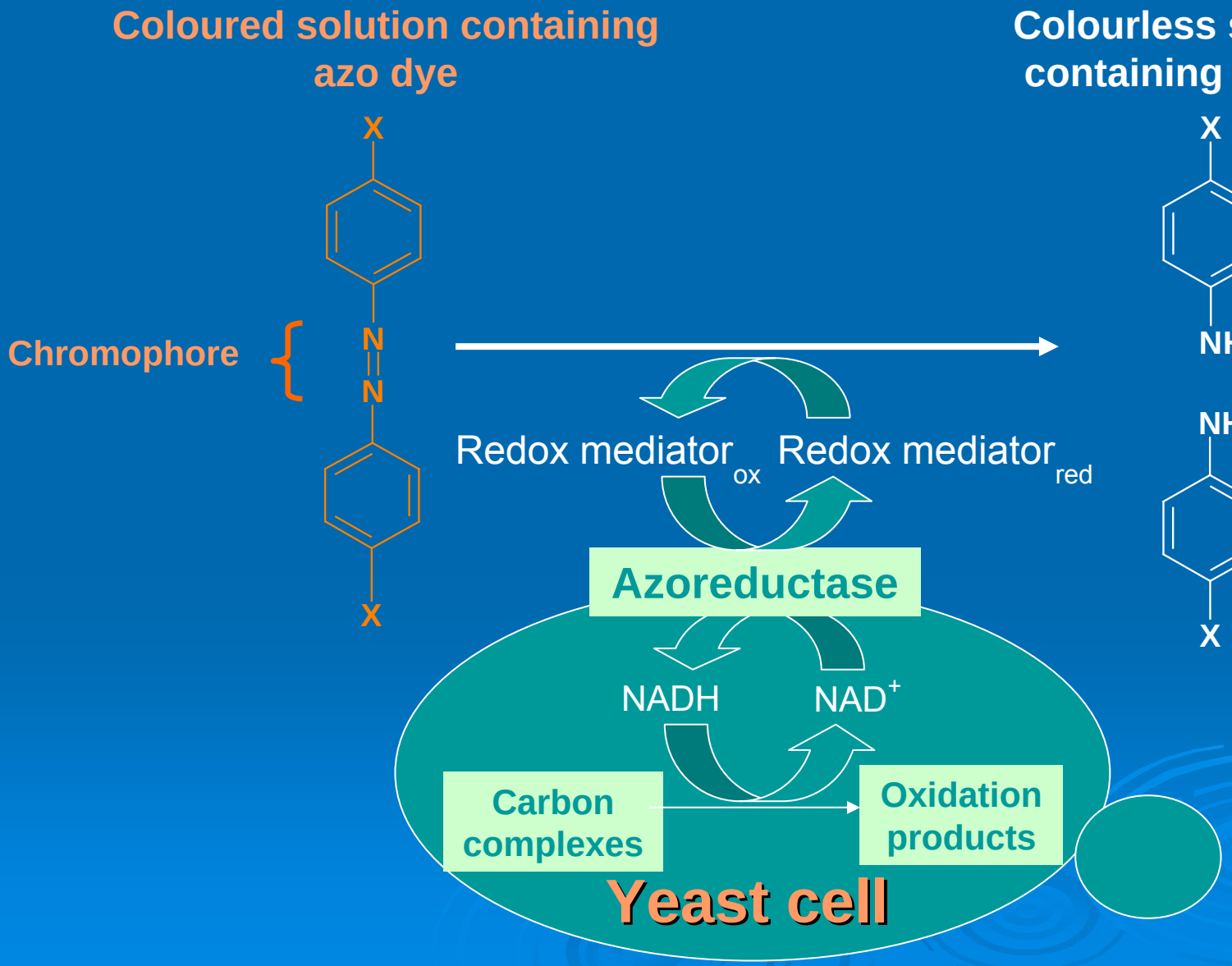
- Isolated from contaminated soil
- k_c = 0,31 h^{-1}
- $Y_{biomass}$ = 16,8%
- $t_d \approx$ 15 h

Strain UM41:

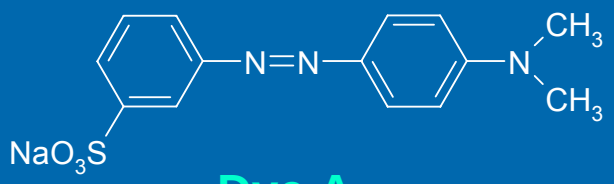
Issatchenkia occidentalis



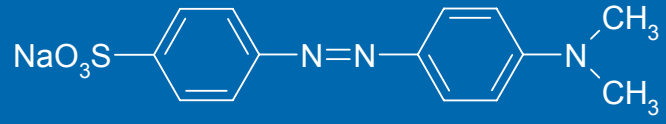
The azo reduction by yeasts



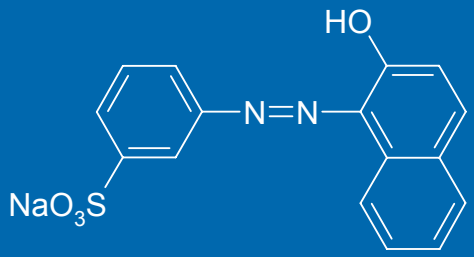
Structures of the tested dyes



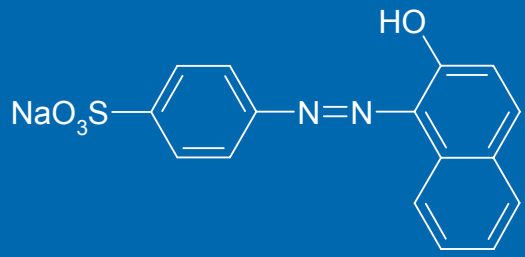
Dye A



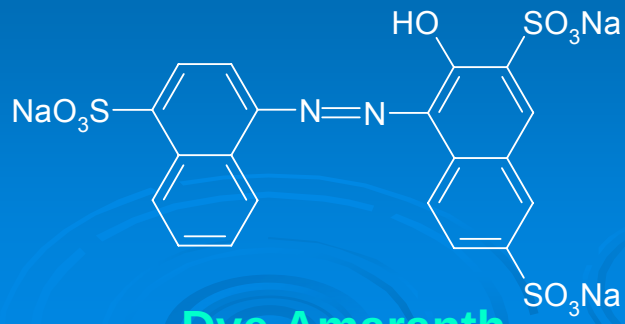
Dye B (Methyl Orange)



Dye C

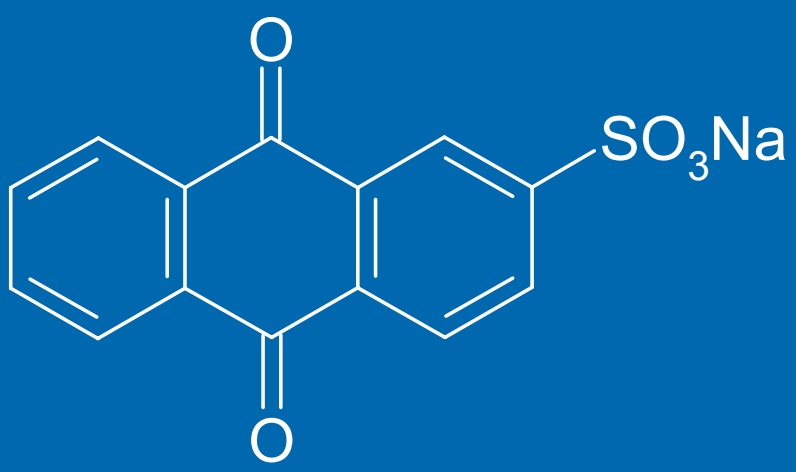


Dye D (Orange II)

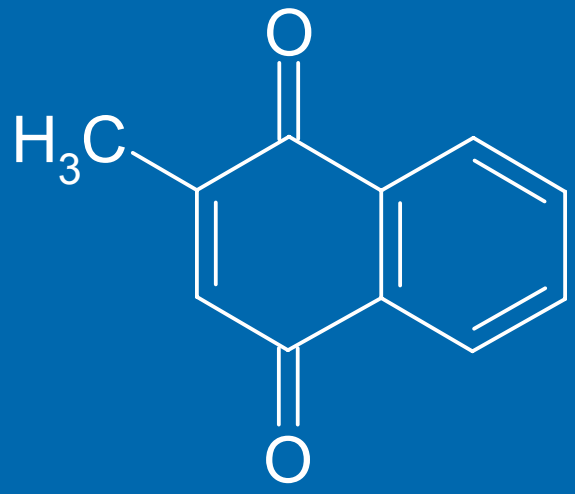


Dye Amaranth

The redox mediators: structures



AQS
(9,10-Anthraquinone-2-sulfonate)

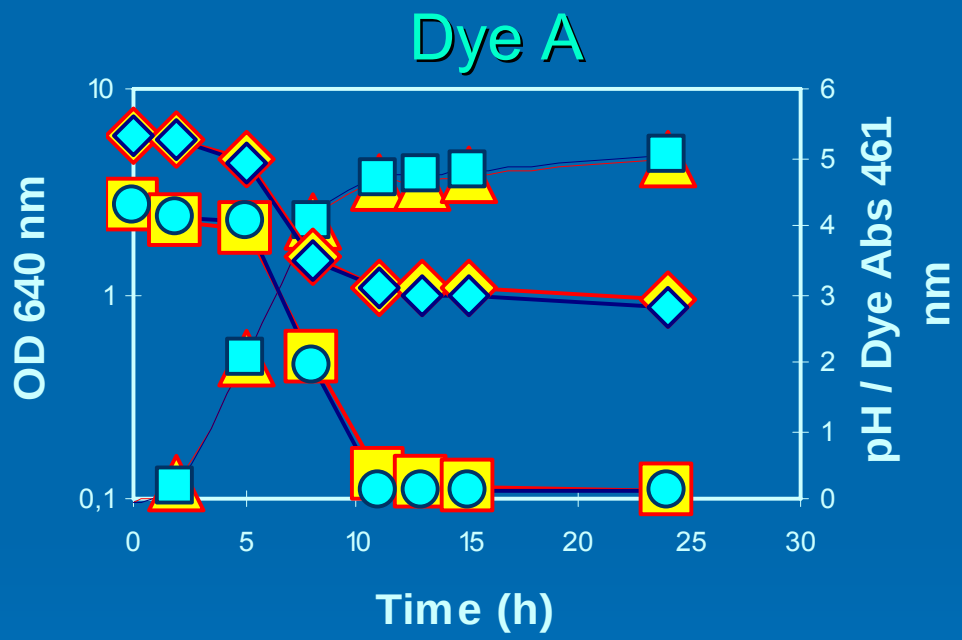


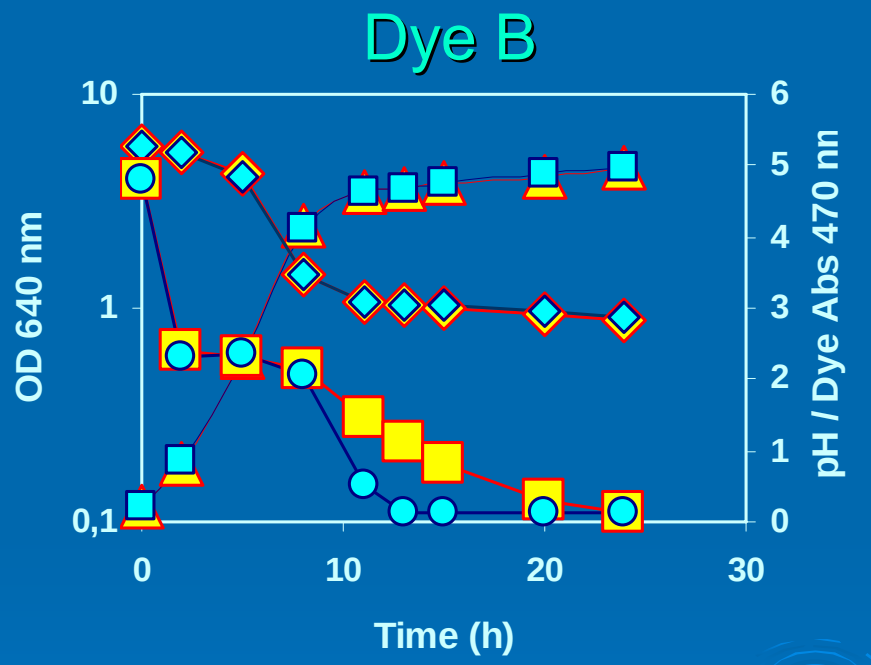
Menadione
(2-Methyl-1,4-naphtoquinone)

Results

1. Decolorization with growing cultures + AQS
2. Resting cells assays: effect of diferent mediators
3. Effect of menadione
4. Cyclic voltammetry

Decolorization with growing cultures + AQS





OD W/o

OD W/

pH W/o

pH W/

Dye Abs W/o

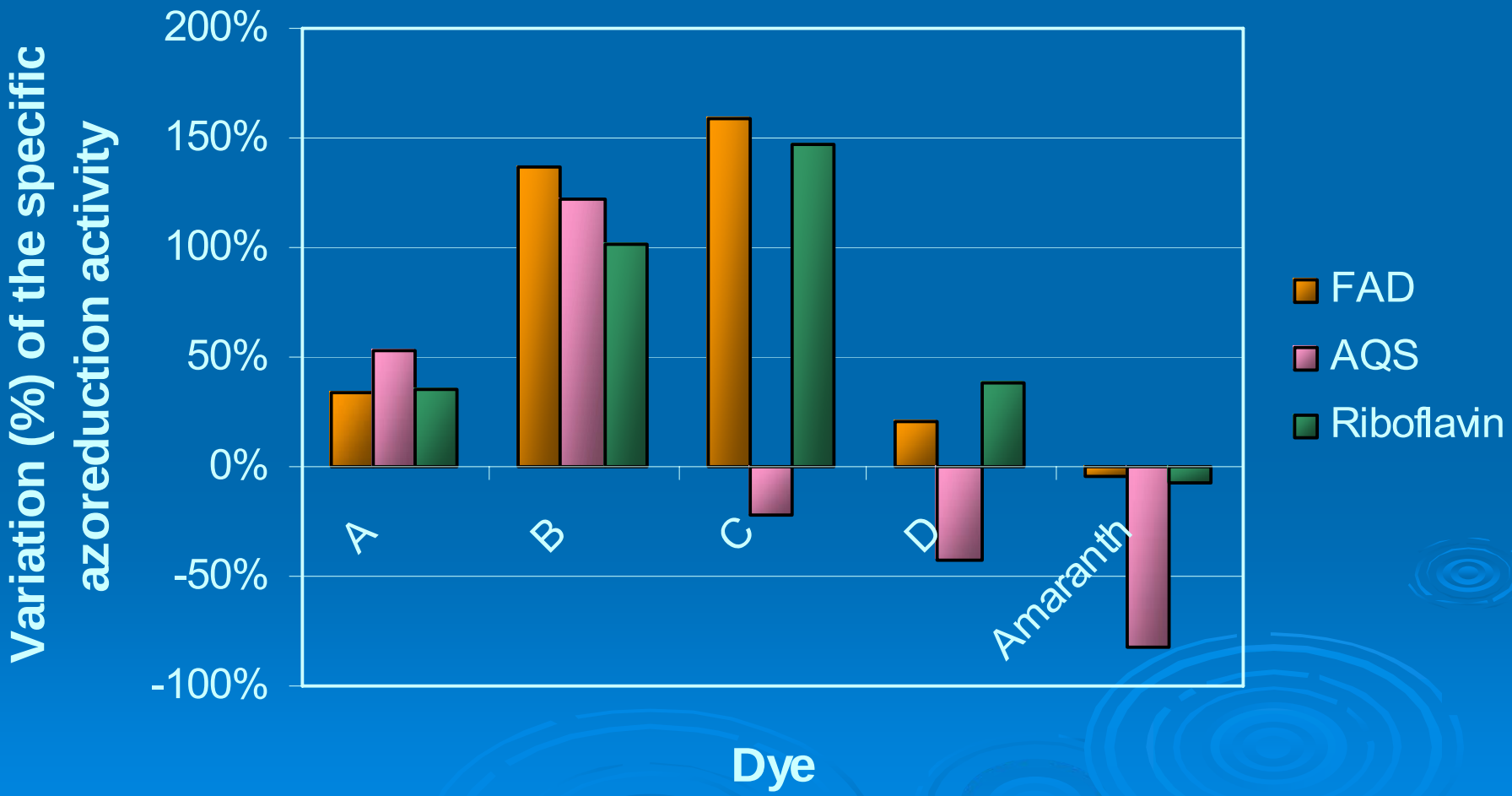
Dye Abs W/

Resting cells assays

- High cell density (c.a. 4 $gDW.L^{-1}$);
- Low glucose concentration (0.011 M);

Measurement of the **azoreductase activity** in different conditions ($\mu mol/(h*gDW)$)

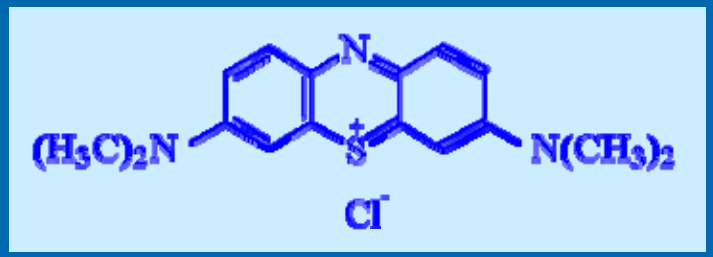
Effect of different mediators: resting cells assays



Oxygen is present in the assays?

Oxygen can compete with the dye for the electrons.

Methylene Blue



Why Menadione?

Menadione enhances the reduction of extracellular electron acceptors by yeast cells.

Baronian et al. *Appl Microbiol Biotechnol* (2002) 60: 108-113

Modification of the “resting cells” assays

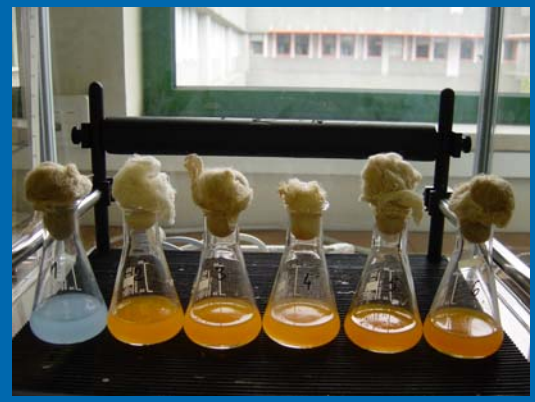
Decrease of the glucose concentration (0.056mM) to minimize growth during the assay.

Menadione – standard conditions

I – Before dye addition

II – After dye addition

III – End of the assay (2h)



1 – Methylene Blue + Cells
2 – Dye D + Cells
3 – Dye D + Cells + AQS
4 – Dye D + Cells + AQS + Menadione
5 – Dye D + Cells + FAD
6 – Dye D + Cells + FAD + Menadione

No significant effect !

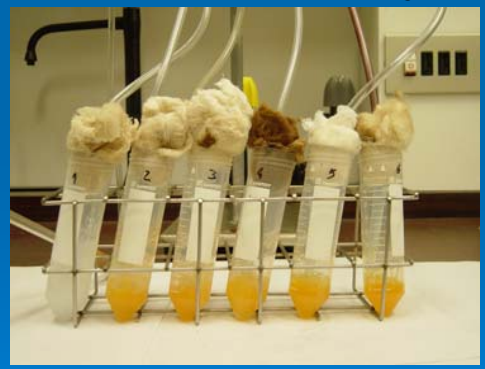
Menadione – without O_2

I – Before N_2 sparging

II – Before dye addition

III – After dye addition

IV – End of the assay (2h)



1 – Methylene Blue + Cells
2 – Dye D + Cells
3 – Dye D + Cells + AQS
4 – Dye D + Cells + AQS + Menadione
5 – Dye D + Cells + FAD
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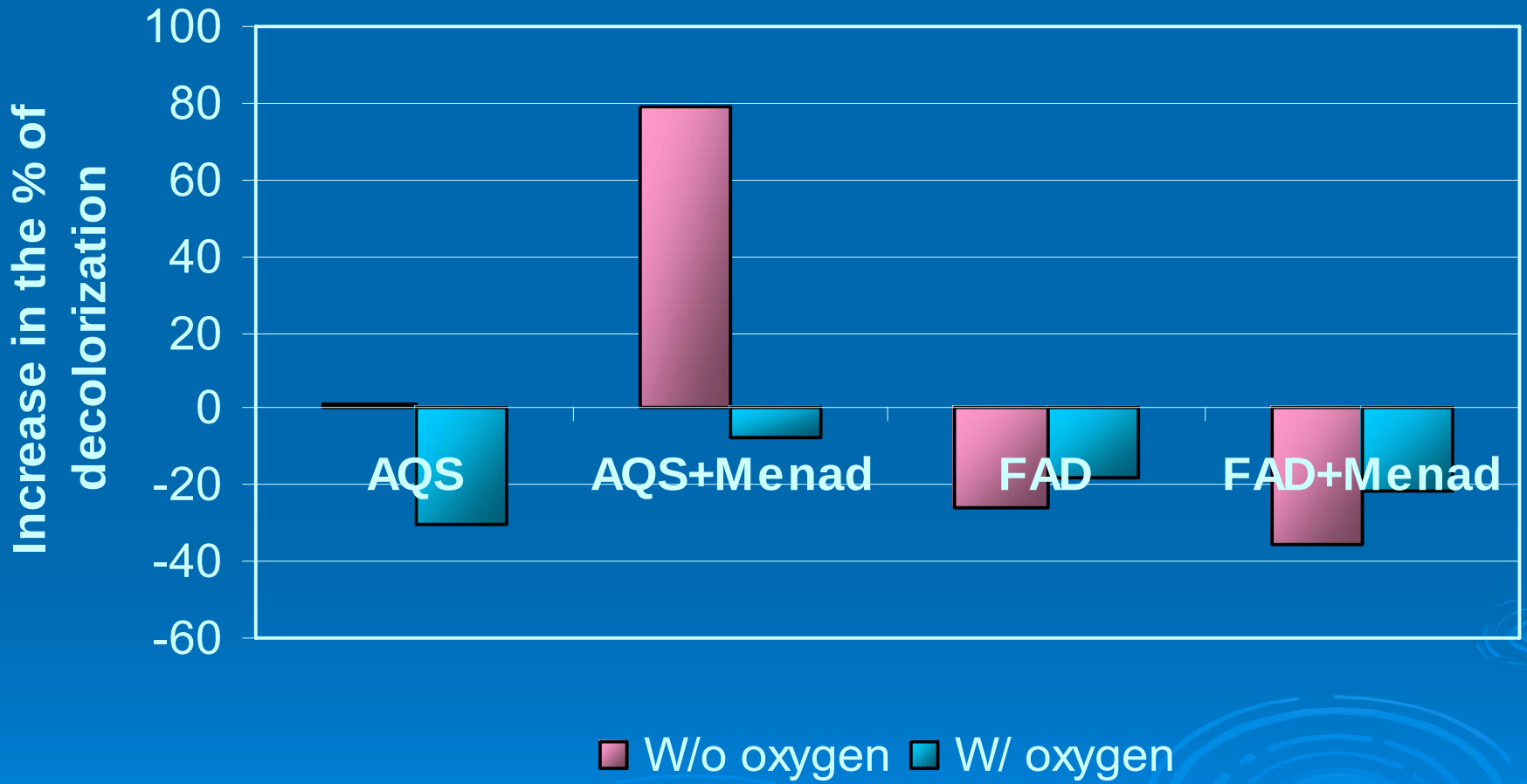
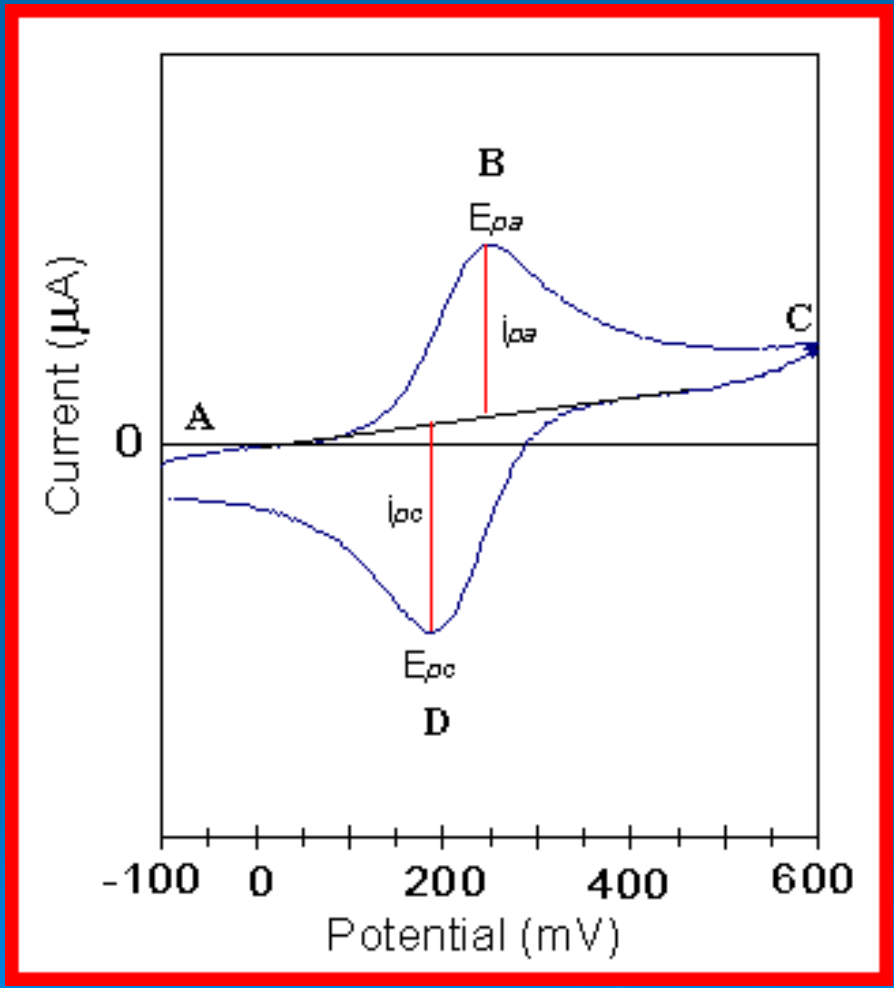
Menadione: results



Cyclic Voltammetry



A – Reduced form
B – Oxidation
C – Oxidized form
D – Reduction

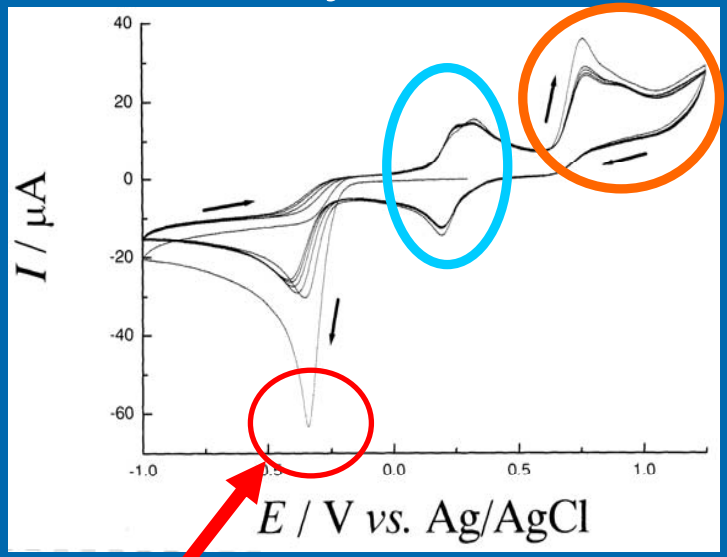
E_{pa} – anodic peak potential
E_{pc} – cathodic peak potential

Formal potential:

$E^{0'} = (E_{pa} + E_{pc}) / 2$

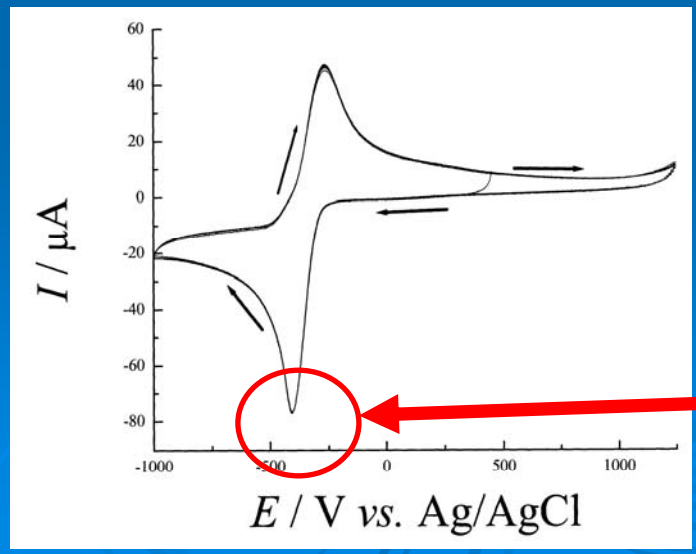
Sample voltammograms

Dye B



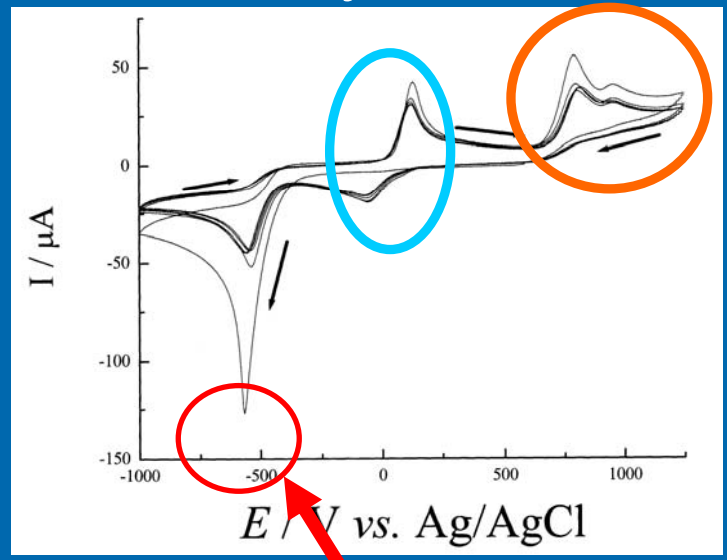
-0.337 V

AQS



-0.398 V

Dye D



-0.560 V

Cyclic Voltammetry: results

Dye	Reduction peak (V)	Decolorization time (h)
A	-0.397	15
B	-0.337	12
C	-0.521	24
D	-0.560	30
Amaranth	-0.476	17

Mediator	Reduction peak (V)
AQS	-0.398

Cathodic peak potentials (literature):

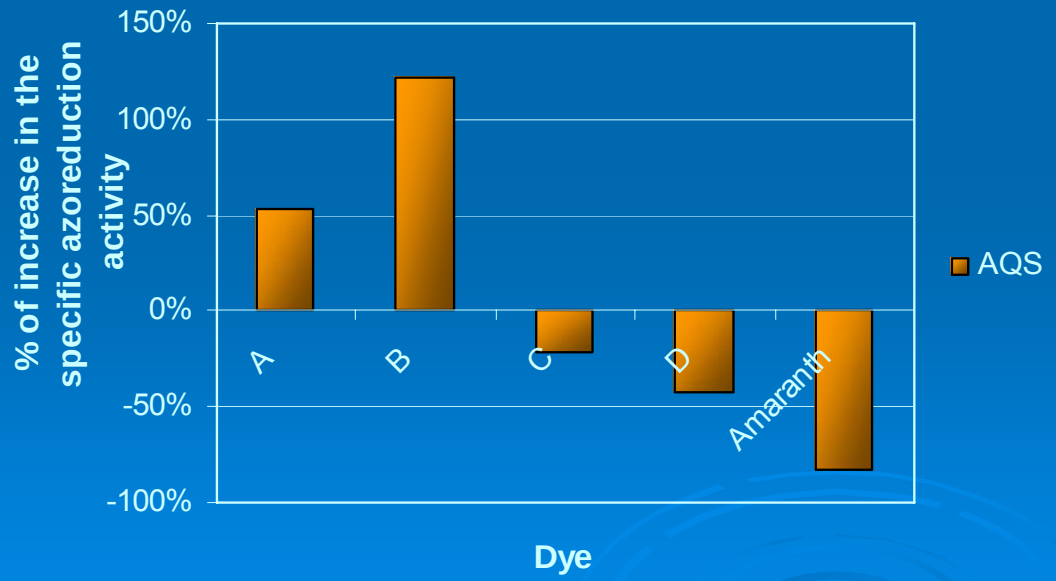
NAD^+ = -0.530 to -0.590 V

FAD / Riboflavin ≈ -0.46V

Conclusions

1. Thermodynamics is crucial.

$$E_{pc_{mediator}} < E_{pc_{dye}}$$



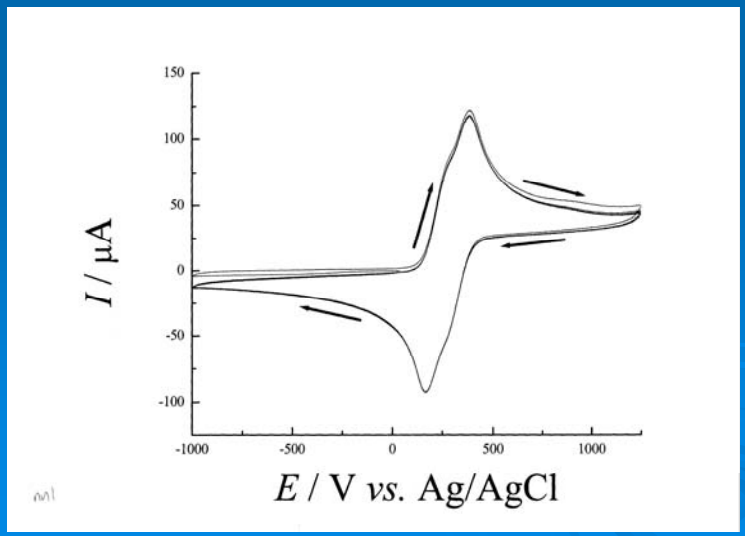
Substance	E_{pc} (V)
A	-0.397
B	-0.337
AQS	-0.398
Amaranth	-0.476
C	-0.521
D	-0.560

Conclusions

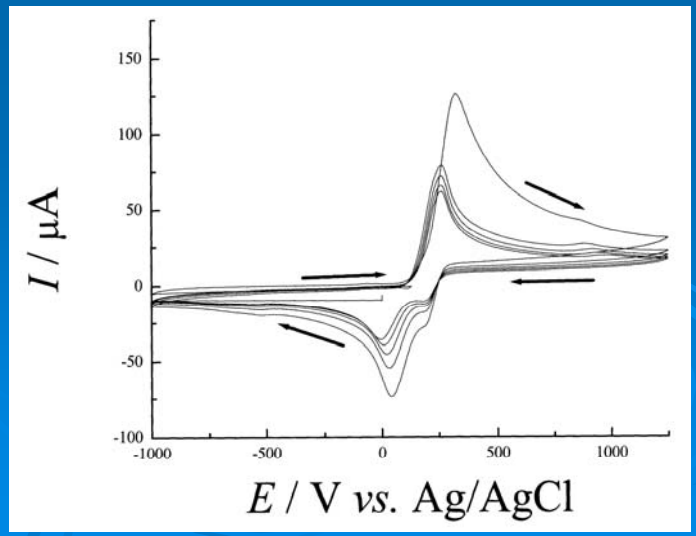
2. The amines N,N-dimethyl-p-phenylenediamine and 1-amino-2-naphthol can not act as mediators because in this case

$$E_{pc_{amines}} > E_{pc_{dye}}$$

N,N-dimethyl-p-phenylenediamine

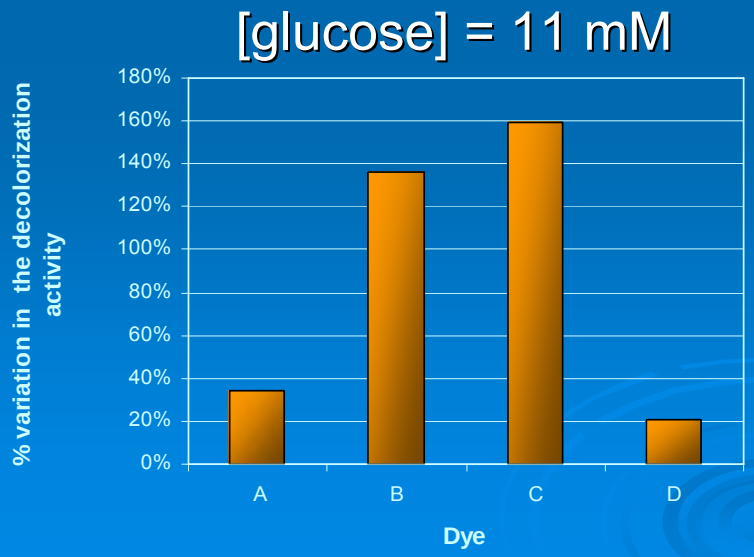


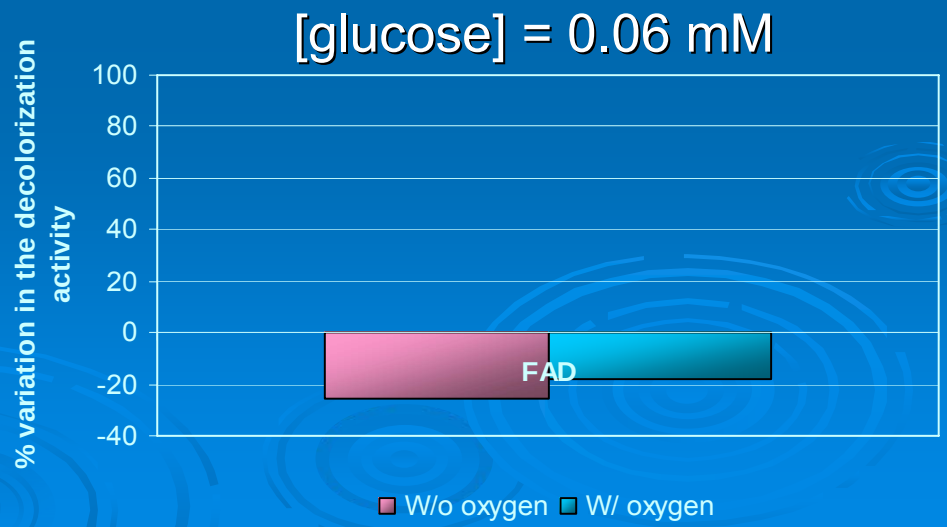
1-amino-2-naphthol



Conclusions

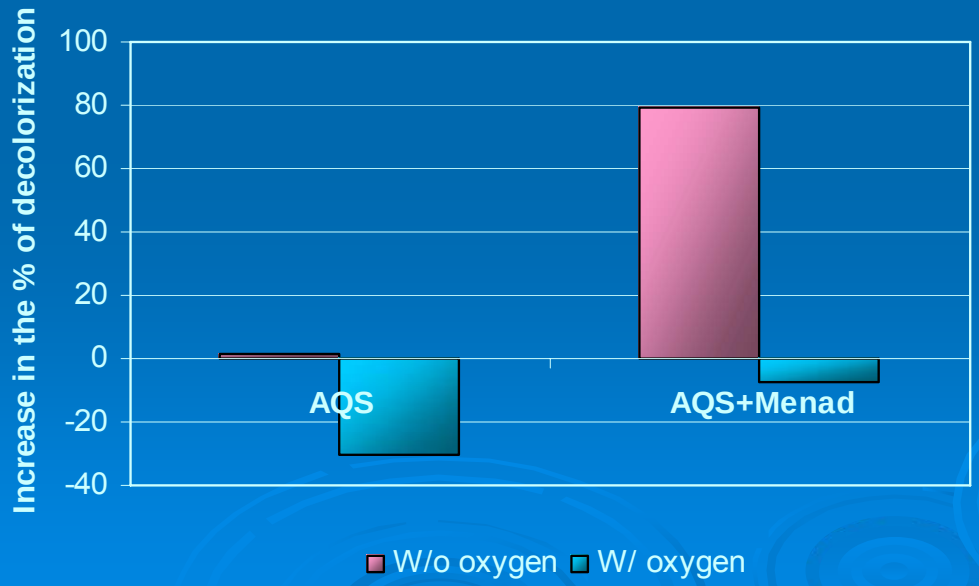
3. The reduction capacity should be dependent on intracelular NAD(P)H concentration, since a decrease in glucose concentration in the media causes an inversion on the effect of the mediator FAD (thermodynamic effect)





Conclusions

4. Menadione has a positive effect mainly in the absence of oxygen. This reinforces our idea of the involvement of a membrane system in azoreduction by yeast cells.



Final conclusions

Cyclic voltammetry is a powerful instrument that can help us to predict:

- **the effect of redox mediators on the biological reduction of azo dyes;**
- **the possibility of biological reduction of this class of compounds not only by yeasts but also by other microorganisms.**