Enzymatic decolourisation of azo and anthraquinonic dyes with CotA- laccase from *Bacillus subtilis*

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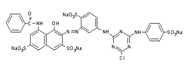
Introduction & Objectives

Azo and antraquinonic dyes have a wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries. These are the largest and most versatile classes of dyes used, but are recalcitrant to biodegradation and many are carcinogenic or cytotoxic.

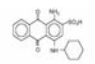
Decolourisation and dye removal using a biotechnological approach remains technically attractive and the development of a laccase based system is quite promising due to the high relative non-specific oxidation capacity of these enzymes. Laccases are able to catalyse the oxidation of synthetic dyes directly or through the use of redox mediators, by reduction of molecular oxygen to water.

In this work, purified recombinant CotA-laccase from *Bacillus subtilis* was tested on its ability to degrade azo and anthraquinonic dyes in the presence and absence of different redox mediators.
Decolourisation of Reactive Black 5 by using intact or alginate immobilized *Escherichia coli* overproducing the CotA-laccase were also conducted.

Textile dyes tested

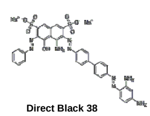


Reactive Red 4 (RR4)



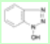
Acid blue 62 (NY3)

Reactive Black 5 (RB5)

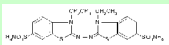


Direct Black 38 (DB38)

Laccase-Mediators used

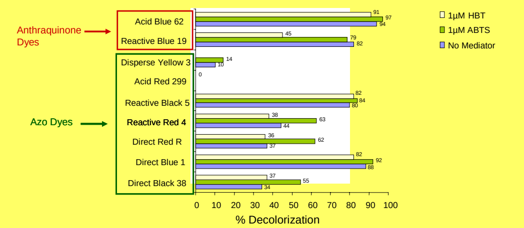


Hydroxybenzotriazole (HBT)



ABTS

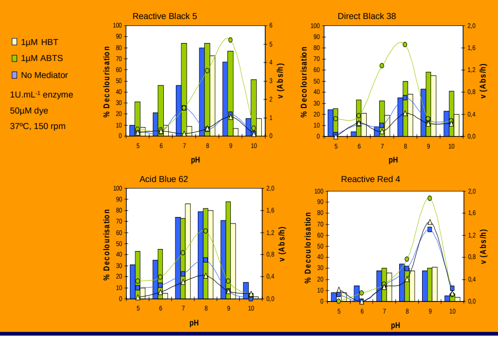
Dye decolourisation by CotA-laccase



50µM Dye,10h of reaction at pH 8 and 37ºC

Except Acid Red 299, all the dyes tested were, at diferent extent, oxidatively bleached by 1U.mL^{-1} CotA-laccase in the absence of mediators.

Effect of pH on dye decolourisation by CotA-laccase



Decolourisation was shown to be **pH-dependent**, being **maximal at the alkaline range** of pH

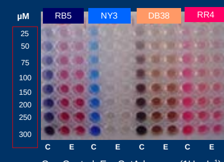
Decolourisation proceeds following a **first order kinetics**

Maximal rates in the presence of ABTS, with an increase of 4.5 fold for RB5 and ~ 2 for NY3, DB38 and RR4 comparatively with the reaction in absence of mediators

Extension of dye degradation at the equilibrium independent of the presence of mediators

Kinetic constants of CotA laccase towards dyes decolourisation

	K_m (µM)	k_{cat} (min^{-1})	E^0_{ox} (mV)
Reactive Black 5	176 ± 2	0.43	742.9
Acid Blue 62	224 ± 6	0.45	788.2
Reactive Red 4	168 ± 9	0.95	975.2
Direct Black 38	29 ± 2	0.34	636/1021



C – Control; E – CotA-laccase (1U.mL^{-1})

Reaction at 37ºC, pH 8.0

K_m and k_{cat} were calculated assuming simple Michaelis-Menten kinetics

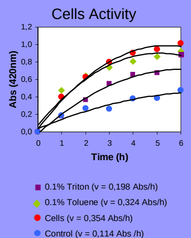
The redox potentials of the dyes were measured by cyclic voltammetry
Work done in collaboration with Cavaco-Paulo group (Textile Department, Minho University, Portugal)

No obvious correlation between the values of k_{cat} and the redox potential measured for the dyes was found

Reactive Black 5 decolourisation

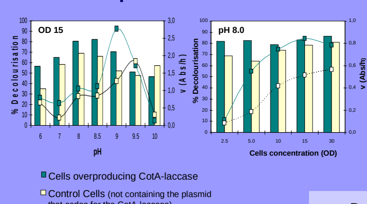
Whole Cell Catalysis

Reducing cell permeability



	Supernatant	
	Protein (mg/mL)	Activity (nomo/min.m
0.1% Toluene (30ºC, 30min)	0.12	4.2
0.1% Triton (30ºC, 30min)	0.18	5.1
No addition (30ºC, 30min)	0.04	6.3
Control	0.006	7.0

Effect of pH and cell concentration



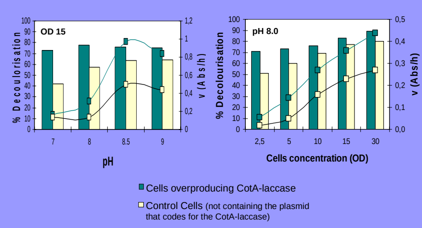
Cells overproducing CotA-laccase
Control Cells (not containing the plasmid that codes for the CotA-laccase)

24h reaction at 37º, 150 rpm

Immobilized Cell Catalysis

Dye decolourisation due to:
- enzymatic action (~20%)
- adsorption to cells (~80%)

Effect of pH and cell concentration

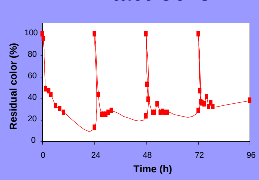


Cells overproducing CotA-laccase
Control Cells (not containing the plasmid that codes for the CotA-laccase)

24h reaction at 37º, 150 rpm

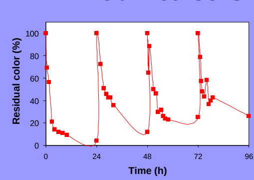
Decolourisation in a continuous addition system 500µM dye

Intact Cells



37ºC, 150 rpm, OD 15, pH 9.0

Immobilized Cells



37ºC, 150 rpm, OD 15, pH 8.5

Intact Cells *vs* Immobilized cells

	Decolourisation (%)		v (Abs.h^{-1})	
	Whole cells	Immobilized cells	Whole cells	Immobilized cells
First cycle	88 ± 0.79	96 ± 0,0	0.67 ± 0.09	0.42 ± 0.01
Second cycle	77 ± 1.19	89 ± 1.0	1.17 ± 0.44	0.41 ± 0.02
Third cycle	71 ± 1.24	83 ± 8.0	1.44 ± 0.06	0.49 ± 0.07
Fourth cycle	62 ± 2.11	78 ± 4.0	2.16 ± 0.07	0.31 ± 0.02