Microbial degradation of azo dyes by *Pseudomonas putida* MET 94 strain

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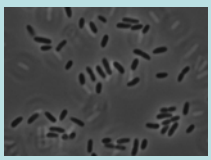
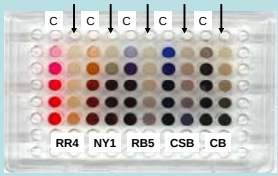
Introduction

Azo aromatic dyes are the major group of textile dyestuffs. These are chemically stable structures to meet various colouring requirements and often are not degraded and/or removed by conventional physical and chemical processes. Moreover, many of these compounds are highly resistant to microbial attack and therefore, they are highly visible and hardly removed from effluents by conventional biological processes such as activated sludge treatment. Over the last two decades, considerable work has been done with the goal of using microorganisms as bioremediation agents in the treatment of wastewater containing textile dyes. In most of the reported processes of azo dye biodegradation, however, the participation of an enzymatic system is assumed.

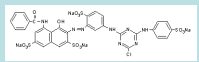
In this work, we have examined the decolourisation activity of a *Pseudomonas putida* strain MET94 selected among 84 bacterial strains has the most active textile dye degrader

P. putida MET94 strain & Dyes

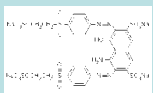




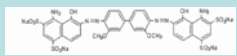
Reactive Red 4	RR4
Acid Red 299	NY1
Reactive Black 5	RB5
Direct Blue 1	CSB
Direct Black 38	CB



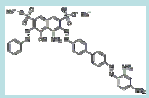
Reactive Red 4 (RR4)
Cibacron Brilliant Red 3B-A



Reactive Black 5
(RB5)



Direct Blue 1
(CSB)

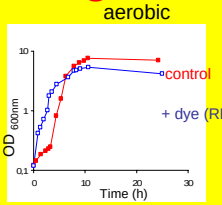


Direct Black 38
(CB)

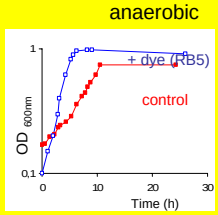
Aerobic and anaerobic degradation by growing cells

Aerobic and anaerobic degradation of dyes at 30ºC in LB medium supplemented with 150µM of dye

aerobic

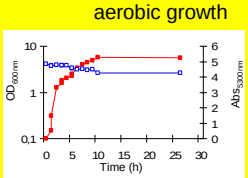


anaerobic

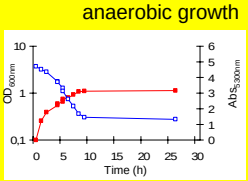


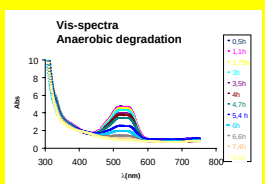
Degradation of reactive red 4 (RR4)

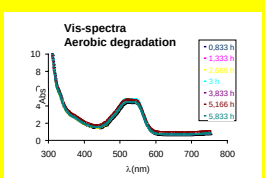
aerobic growth



anaerobic growth







Growth rates in the absence and presence of dyes

Dyes exerting toxicity over cell growth at aerobic conditions?

Growth rates (h^{-1})	Aerobic	Anaerobic
Control	0.64	0.14
RB5	0.51	0.29
RR4	0.25	0.20
CSB	0.41	0.07
CB	0.34	0.20

Dyes used as electrons acceptors in anerobic respiration?

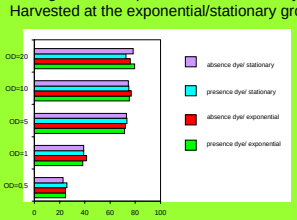
Decoulorization occured at higher extent and rates at anaerobic conditions of growth

	Aerobic		Anaerobic	
	Decoul (%)	rate (h^{-1})	Decoul (%)	rate (h^{-1})
RB5	68	0.08	74	0.14
RR4	19	0.01	72	0.12
CSB	26	0.04	85	1.02
CB	10	0.02	67	0.15

Resting Whole-cell degradation

Degradation of RB5

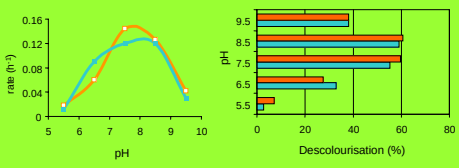
Cells grown in the presence/absence of dyes
Harvested at the exponential/stationary growth phase



	Aerobic				Anaerobic			
	presence dye		absence dye		presence dye		absence dye	
	rate (h^{-1})	Dc. (%)	rate (h^{-1})	Dc. (%)	rate (h^{-1})	Dc. (%)	rate (h^{-1})	Dc. (%)
pH=8.5	0.18	86.14	0.24	83.0	0.20	89.7	0.61	97.0

Optimal decolourisation at pH 7.5 – 8.5

OD 10, aerobic conditions



Conclusions

•Higher degradation rates as well as higher extent of decolourization were obtained in anaerobic when compared with aerobic conditions.

• In the absence of oxygen (i) degradation is growth associated, (ii) specific growth rates were higher in the presence of dyes, suggesting that these could be used as electron acceptors in anaerobic respiration.

• In the presence of oxygen (i) growth rates as well as biomass yields were lower in the presence of dyes, suggesting that dyes could be exerting toxicity over cells, (ii) maximum decolourization activity occurred at the late exponential-stationary growth phase.

•Both in aerobic and in anaerobic conditions the enzymatic catabolic system employed is constitutive as growth initiated by adapted and nonadapted innocula did not present any significant difference. Culture supernatants were unable to decoulorize the dyes tested.

•Kinetic assays of RB5 and DB1 degradation were performed with whole-cells grown aero and anaerobically in the presence and absence of dyes. These studies demonstrated that: (i) rates and degree of decoulorization were not dependent on cells being grown in the presence of dyes but depends strictly on biomass concentration on reaction assays, (ii) optimal pH range for degradation was in basic range.