Microaerophilic–aerobic sequential decolourization/biodegradation of textile azo dyes by a facultative *klebsiella* sp. strain VN-31

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Background

To overcome the accumulation of toxic aromatic amines in reductive azo dye biodecolourization, recent studies included combinations of anaerobic and aerobic steps in an attempt to achieve their degradation. However, very few studies have been performed using sequential microaerophilic/aerobic conditions with the same microorganism.

Objectives

1. Azo dye decolourization under reductive conditions using a facultative *Klebsiella* sp. strain VN-31.
2. Biodegradation by stirring aeration to promote aromatic amines oxidation into non-toxic metabolites.

Methods

Strain identification was performed by 16S rRNA gene sequence analysis. Dye decolourization and degradation products were studied by direct measures, UV-Vis and FT-IR analysis. Total Organic Carbon was measured by TOC analyzer. Acute toxicity tests were carried using D*aphnia magna.*

Results

The successive microaerophilic/aerobic stages, using a single *Klebsiella* sp. strain VN-31 in the same bioreactor, were able to reductively decolourize four azo dyes (>94%) and to oxidize the formed aromatic amines into non-toxic metabolites when the medium was aerated. Some differences in the decolourization time depending on the dye structure were confirmed by UV-Vis analysis. The disappearance of aromatic amines during the aerobic stage was confirmed by direct measurement and by FT-IR analysis. No mortality in *Daphnia magna* was detected (except for RR198) in the aerated samples. TOC reduction was ~50% in the microaerophilic stage and ~80% in the aerobic stage for all the dyes. In the aerobic stage partial mineralization of the dye degradation products and of the medium metabolites, was confirmed by the FT-IR, toxicity and TOC measurements.

Conclusions

In a single bioreactor with a single bacterium, only changing the agitation conditions, it was possible not only to decolorize the dyes, but also to achieve a good degree of mineralization and low toxicity, with low running and maintenance costs.

Microaerophilic–aerobic sequential biodegradation of azo dyes by a facultative *Klebsiella* sp. Strain VN-31

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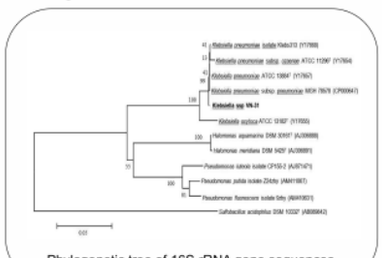
INTRODUCTION

Among the dyes discharged by textile-processing industries, the azo dyes are the most important chemical class of synthetic dyes and pigments [1]. In addition to their visual effect and their adverse impact in terms of BOD, COD, heat, pH and metal ions, many synthetic dyes are toxic and mutagenic [2]. In recent years, new processes for dye degradation and wastewater reutilization have been developed [3]. In particular, systems based on biological processes using a large variety of bacterial strains, allow for degradation and mineralization under mild pH and temperature conditions [4]. Amongst these systems, several facultative anaerobic bacterial strains have been described as being capable of reducing azo dyes [5]. Reductive azo dye decolourization by microorganisms usually leads to the accumulation of toxic aromatic amines [6]. To overcome this problem, recent studies included combinations of anaerobic and aerobic steps in an attempt to achieve dye decolourization and amine degradation [7]. However, very few studies have been performed using sequential microaerophilic/aerobic conditions with the same microorganism, preferring the use of consortia or different microorganisms, used separately under anaerobic, microaerophilic and aerobic conditions [8].

In this study, degradation of four azo dyes was carried out under microaerophilic conditions until no colour was observed using a facultative *Klebsiella* sp. strain VN-31. The medium was then aerated by stirring to promote oxidation of the aromatic amines into non-toxic metabolites. The degradation products were characterized by FT-IR and UV-Vis techniques and their toxicity and Total Organic Carbon (TOC) measured.

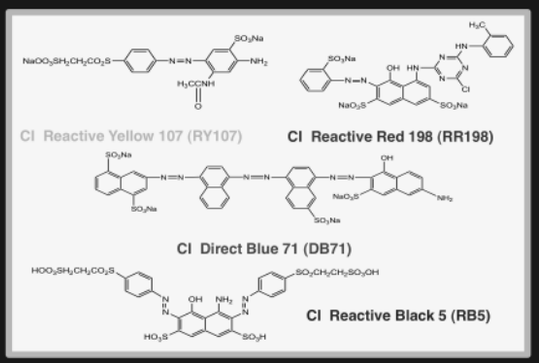
BIOLOGICAL MATERIAL

The phylogenetic tree of the partial sequences based on the 16S rRNA gene of the *Klebsiella* sp. strain VN-31 (activated sludge, textile company, Brazil) was constructed by the neighbour joining method. The bootstrap and values higher than 70 % were indicated on the tree. *Sulfobacillus acidophilus* DSM 10332^T was used as the outgroup. The nucleotide alignment supported values of the boot strap of 99% similarity to *Klebsiella pneumoniae subsp pneumoniae* and other *Klebsiella* sp.



Phylogenetic tree of 16S rRNA gene sequences of *Klebsiella* sp.

RESULTS & DISCUSSION



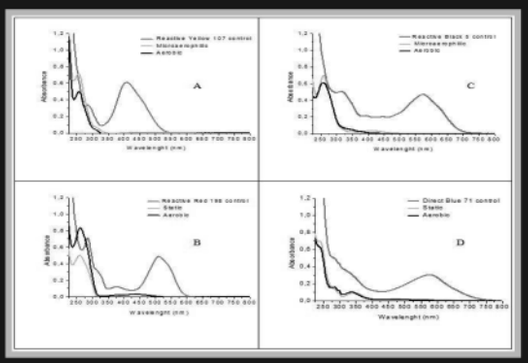


FIG. 1 & 2. Chemical structures and UV-Vis spectra of the azo dyes before (straight line) and after microaerophilic (dashed line) and aerobic (dotted line) treatments - A: RY107; B: RR198; C: RB5; D: DB71.

- After biodegradation the peaks in the visible region disappeared, indicating complete decolourization.
- The absence of the typical absorption peak of the hydrogenated azo bond structure (Ar---NH---NH---Ar') at 245 nm in all the dyes indicated complete disruption of the azo bonds.
- The decrease in absorbance of the peaks at 285 and 320 nm, related to the benzene and naphthalene rings, respectively, and the formation of a new peak at 260 nm, uncovered the fine multi-peaks of aromatic rings.

	Amine concentration (mM)		Decolourization time (h)	Decolourization (%)	
Dyes	**Microaerophilic**	**Aerobic**	**Microaerophilic**	**Microaerophilic**	**Aerobic**
RY107	0.16 ± 0.04	0.01 ± 0.02	72 ± 4	100 ± 0.1	92.8 ± 0.5
RB5	0.24 ± 0.02	0.01 ± 0.03	120 ± 8	94 ± 0.6	92.8 ± 0.3
RR198	0.1 ± 0.03	0.02 ±0.02	96 ± 5	98 ± 0.5	100 ± 0.1
DB71	n.d.	n.d.	168 ± 12	94 ± 0.4	96.6 ± 0.4

n.d. – not detected

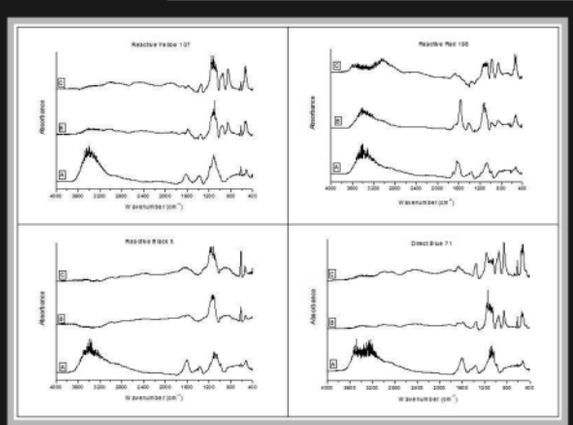


FIG 3. FT-IR spectra of the azo dyes before (A) and after microaerophilic (B) and aerobic (C) treatments.

- The bands within 1610-1630 cm^{-1} and at 1402 cm^{-1} (–N=N– on aromatic structures and of –N=N– stretching in α-substituted compounds) diminished in the microaerophilic and aerobic stages.
- In the microaerophilic stage two bands in the carbonyl region at around 1680-1600 cm^{-1} appear (amide derived from ammonia or primary amine).
- In the aerobic stage, these two bands disappeared and a new peak appeared at 1680 cm^{-1} (carbonyl group in a carboxylic acid, ketone, ester or conjugated aldehyde group attached to an aromatic ring).
- The new broad region between 2300 and 2500 cm^{-1} in the aerobic stage (carboxylic acid and NH_3^+ ions) suggesting a partial mineralization. After biodegradation the peaks in the visible region disappeared, indicating complete decolourization.

	Daphnia magna mortality (%) *			TOC reduction (%)**	
Dyes	**Control**	**Microaerophilic**	**Aerobic**	**Microaerophilic**	**Aerobic**
RY107	47	33	0	56	78
RB5	47	40	0	46	74
RR198	47	27	10	54	64
DB71	53	60	0	51	87

*SD ± 11% for all the data; **SD ± 2% for all the data

CONCLUSIONS

- All the dyes tested were totally decolourized (>94%) under microaerophilic conditions forming amines by a 16S RNA gene identified *Klebsiella* sp.
- In the aerobic stage, oxidation of the amines and partial mineralization of the dye degradation products were confirmed by the direct measures, FT-IR, toxicity (*Daphnia magna*) and TOC.
- In a single bioreactor with a single bacterium, only changing the agitation conditions, it was possible not only to decolourize the dyes, but also to achieve a good degree of mineralization and low toxicity, with low running and maintenance costs.

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Funding sources and acknowledgements.

The authors would like to thank the Portuguese Foundation of Science and Technology (FCT) for providing the grant to Andrea Zille (SFRH/BPD/24238/2005), the Brazilian Foundations for the Coordination of Training Graduated Pessoal of the Ministry of Education (CAPES) and the National Counsel for Technological and Scientific Development (CNPq) for providing the grant to Elisangela Franciscon Dias.

This poster is based on the following publication:

E Dias-Franciscon, **A Zille**, F Dias Guimaro, C Ragagnin de Menezes, L R Durrant, A Cavaco-Paulo (2009) *Process Biochemistry*. 44:446-452.