Short communication

Anaerobic decolorisation of reactive dyes in conventional sewage treatment processes

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Abstract

Reactive dyes have been identified as problematic compounds in textile effluents as they are water-soluble and not removed by traditional aerobic biological waste-water treatment systems. The use of anaerobic digestion for the decolorisation of selected reactive dyes was investigated. It was found that 80 % of the dyes studied were decolorised and, based on the results from a detailed study into C.I. Reactive Red 141 decolorisation, it was proposed that this occurred via a reduction mechanism. This was supported by the tentative chemical identification of the dye degradation products.

Introduction

Waste water from textile industries is highly coloured and of a complex and variable nature. Conventional biological treatment processes presently in use at waste-water treatment works (i.e. aerobic systems such as activated sludge and trickling filters) do not usually achieve satisfactory colour removal, resulting in coloured effluent entering the receiving water body. This gives rise to complaints, either due to aesthetic reasons, or because it precludes some downstream use of the water. Pollution prevention, waste minimisation and closed loop recycle of water and chemicals are the initial stages in the treatment of textile effluents. The treatment of concentrates has still to be addressed. In particular, reactive dyes have been identified as the most problematic compounds in textile effluents as they are difficult to remove due to their high water solubility and low exhaustion (between 10 and 50 % of the dye will be present in the dyebath effluent) (ENDS Report, 1993). Reactive dyes remain in solution due to their hydrophilic nature and will therefore pass through the aerobic treatment systems in a waste-water treatment works rather than be associated with the solids which are treated in the anaerobic digester. A study of the literature has indicated the potential of anaerobic systems for the non-specific decolorisation of azo dyes (Brown and Laboureur, 1983; Harmer and Bishop, 1992; Kremer, 1989) and therefore an investigation into the feasibility of anaerobic digestion for the decolorisation of a reactive dye, C.I. Reactive Red 141 (Fig. 1), was initiated (Carliell, 1993). It must be noted that not all reactive dyes are based on the azo chromophore and that other dye classes can contain an azo group. Separation and identification of the dye metabolites was also investigated and a reaction mechanism proposed. This decolorisation study was then extended to include other reactive dyestuffs which are representative of the spectrum of dyes used in a dyehouse producing upmarket furnishing and fashion fabric. Based on these results, an on-site study into the fate of C.I. Reactive Red 141 in a 5-stage Bardenpho nutrient removal activated sludge process was carried out at the Hammarsdale Waste-water Treatment Works. In addition, biomass was collected for laboratory-scale studies.

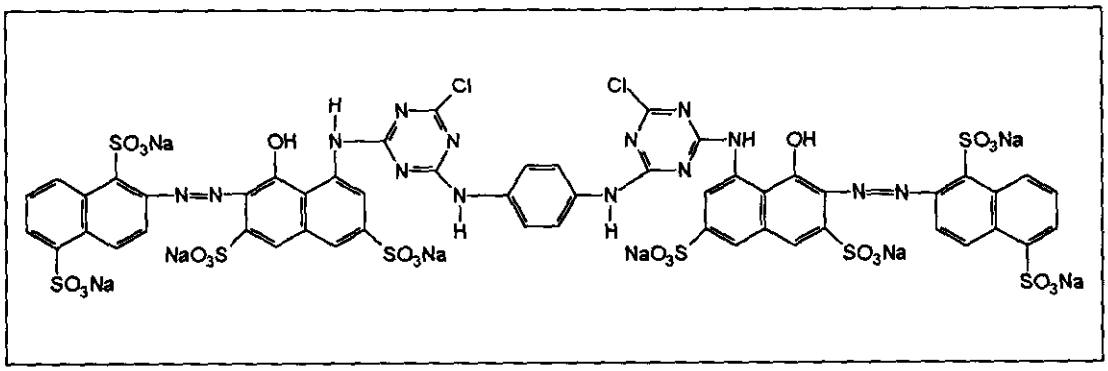

Figure 1
Proposed structure of C.I. Reactive Red 141

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TABLE 1
LIST OF REACTIVE DYES TESTED FOR DECOLORISATION IN AN ANAEROBIC SYSTEM

Dyeing	Printing
C.I. Reactive Yellow 16	C.I. Reactive Yellow 95
C.I. Reactive Red 198a	C.I. Reactive Orange 12
C.I. Reactive Red 141	C.I. Reactive Red 218
C.I. Reactive Blue 38	C.I. Reactive Red 24
C.I. Reactive Blue 21	C.I. Reactive Orange 13
C.I. Reactive Blue 220	C.I. Reactive Brown 11
C.I. Reactive Black 5	* Blue PB
	C.I. Reactive Blue 49
	C.I. Reactive Black 39
	* Black mix
	C.I. Reactive Blue 72

* No C.I. number available.

Materials and methods

Anaerobic studies

Initial studies (Carliell, 1993) showed that decolorisation of a reactive azo dye, C.I. Reactive Red 141, occurred rapidly in an anaerobic test system. A supplemental carbon source was necessary. This work was then extended to include a range of reactive dyes for printing and dyeing (Table 1).

Materials

Assay medium : The assay medium used to conduct the decolorisation tests consisted of glucose (1 g/ℓ) in phosphate buffer (0.025 M, pH 6.9).

Dye preparation : Commercially available dyes were used in this investigation. Concentrated stock solutions of the dyes were prepared in phosphate buffer (pH 6.9). The reactive dyes used for dyeing processes were supplied as powders and dye concentrations of 100 mg/ℓ were studied. The printing dyes were supplied as solutions of unknown concentrations which were diluted approximately 1 000-fold for the study.

Inoculum : Inoculum for the tests was obtained from a laboratory digester seeded with anaerobic sludge from waste-water treatment works receiving textile dyehouse effluents. Organic material in the digester sludge provided the carbon source for digestion.

Reaction vessels : All experiments were performed in serum bottles (120 mℓ) which were sealed with butyl rubber stoppers and aluminium crimp seals.

Method

The serum bottles were inoculated with 30 mℓ of digester sludge and 70 mℓ of assay medium was added. The bottles were over-gassed with oxygen-free nitrogen, sealed and pre-incubated overnight in a water bath at 32°C, without agitation. Thereafter, 2 mℓ of dye stock solution was added to each serum bottle to give dye concentrations of 100 mg/ℓ (for the powder reactive dyes), and approximately 1 000-fold dilutions for the printing reactive dyes. Samples were withdrawn from the serum bottles with a hypodermic needle and syringe, centrifuged to remove suspended solids and analysed colorimetrically at the wavelength of maximum absorbance for each dye tested.

Separation and identification of dye metabolites

The anaerobic metabolites of C.I. Reactive Red 141 were separated and identified using column chromatography, thin layer chromatography (TLC) and proton nuclear magnetic resonance spectroscopy (NMR).

Column chromatography: The liquid samples from the laboratory anaerobic digester were evaporated to dryness and the residue Soxhlet extracted into methanol. The extract was concentrated by rotary evaporation and loaded onto a column packed with Merck 9385 silica gel (230 to 400 Å). A solvent system of methanol-methylene chloride with an increasing ratio from 10 to 90 % (v/v) was used. Fractions were collected, concentrated by evaporation and analysed by TLC.

TLC: Merck 5554 silica gel 60 aluminium backed F_{254} (20 x 20 mm) precoated (0.2 mm) TLC plates were used. A solvent system of 30 % (v/v) methanol-methylene chloride was used. The fractions were spotted on the plate and like fractions identified. These fractions were redissolved in methanol, combined and evaporated to dryness for NMR analysis.

Proton NMR: A Varian Gemini-300 spectrophotometer was used for the analysis. The samples (10 to 20 mg) were dissolved in deuterium oxide (0.75 mℓ) and placed in a sample tube. The tube was suspended in the instrument and spun at 20 r·s^{-1}. Ambient operating temperatures were used.

The fate of C.I. Reactive Red 141 in a 5-stage Bardenpho nutrient removal reactor

A detailed description of the Hammarsdale Waste-water Treatment Works has been described elsewhere (De Haas et al., 1993).

On-site investigation: Dye powder (2.5 kg) was dosed into the incoming effluent as it entered the anaerobic zone and samples were collected at half-hour intervals.

Laboratory investigation: Sludge from the anaerobic and 2 anoxic zones (primary and secondary) of the reactor was placed in 2.5 ℓ bottles and 100 mg/ℓ of C.I. Reactive Red 141 was added. The unstoppered bottles were agitated and samples taken at hourly intervals, centrifuged for 20 min and the absorbance read at 544 nm (wavelength of maximum absorbance). A sample of the sludge prior to addition of the dye was used as a blank.

Results and discussion

It was found (Carliell, 1993) that anaerobic decolorisation of C.I. Reactive Red 141 was dependent on the reduction potential of the solution and that decolorisation occurs after nitrate removal. This suggested that the decolorisation reaction did not depend on the interaction of the bacteria with the dye molecule, but rather the reducing conditions provided by the bacteria (Carliell, 1993).

The serum bottle tests on the range of reactive dyes indicated that 80 % of the dyes were decolorised. The results are listed in

TABLE 2
RESULTS OBTAINED WHEN INCUBATING SELECTED REACTIVE DYES IN AN ANAEROBIC SYSTEM WITH SUPPLEMENTAL CARBON

	Chemical classification	Percentage decolorisation	Reaction time (h) required to attain maximum decolorisation
Reactive dye powders			
C.I. Reactive Yellow 16	azo	80 to 90	6.5
C.I. Reactive Red 198a	azo	85 to 90	2
C.I. Reactive Red 141	azo	85 to 90	4.5
C.I. Reactive Blue 38	phthalocyanine	40	4.5
C.I. Reactive Blue 21	phthalocyanine	85 to 90	4.5
C.I. Reactive Blue 220	azo	90 to 95	1
C.I. Reactive Black 5	azo	80 to 85	4.5
Reactive dye solutions			
C.I. Reactive Yellow 95	azo	0	-
C.I. Reactive Orange 12	azo	90 to 95	23
C.I. Reactive Red 218	azo	90 to 95	32
C.I. Reactive Red 24	azo	90 to 97	32
C.I. Reactive Orange 13	azo	85 to 90	50
C.I. Reactive Brown 11	azo	90	23
* Blue PB	metal complex	98	2
C.I. Reactive Blue 49	anthraquinone	7 to 10	2
C.I. Reactive Black 39	azo	70 to 75	5.5
* Black SG	metal complex	75 to 80	7.5
C.I. Reactive Blue 72	phthalocyanine	25 to 30	50

* No C.I. number available

Table 2 and confirm the findings by Carliell (1993) that decolorisation of azo reactive dyes can be achieved in an anaerobic system with a supplemental carbon source. Decolorisation of the azo dyes ranged from 70 to 97 % with the exception of C.I. Reactive Yellow 95. Although reaction times are included in Table 2, it must be noted that these are directly dependent on the concentration of dye in the system (Carliell, 1993). The azo dye, C.I. Reactive Yellow 95 was not decolorised in this anaerobic system. This has been attributed to possible inhibitory or toxic components in the printing dye. From Table 2, it is evident that the other dyes not significantly decolorised are based on chromophores other than the azo system, i.e. anthraquinone and phthalocyanine. It is thought that these structures are more stable and less susceptible to reduction.

Column chromatography of the anaerobically produced metabolites of C.I. Reactive Red 141 indicated the presence of 4 fragments. Analysis by NMR resulted in 3 of the fragments being tentatively identified as 2-aminonaphthalene-1,5-disulphonic acid (I), 1,7-diamino-8-naphtho-3,6-disulphonic acid (II) and *p*-diamino-benzene (III). The fourth fragment did not indicate the presence of any hydrogen atoms and could therefore not be identified by NMR but was thought to be cyanuric acid (IV). The proposed structures are given in Fig. 2. From these results, it was proposed that under anaerobic conditions the azo bonds in the dye molecule were broken (leading to decolorisation) followed by breaking of the amine linkages between the chromophore and the reactive group and within the reactive group itself (as shown in Fig. 2).

The 5-stage Bardenpho nutrient removal (phosphate and nitrate) reactor system was chosen for an on-site investigation into the fate of dyes in a waste-water treatment plant as it consists of an anaerobic zone and 2 anoxic zones (De Haas et al., 1993). In the anoxic zones denitrification of the effluent takes place, and since the anaerobic studies showed that reduction of nitrate was necessary before the decolorisation of C.I. reactive Red 141 could occur, it was thought that decolorisation could be achieved. After the addition of C.I. Reactive Red 141, complete decolorisation occurred within 30 min. Since it was not certain whether this was due to the breaking of azo bonds or due to dilution, laboratory-scale studies were performed. The results are shown in Fig. 3 and confirm that the decolorisation was due to biological activity.

Conclusion

These results indicate that a nutrient removal process operated under certain conditions (i.e. at a redox potential less than that of nitrate reduction), can achieve decolorisation of dyes based on the azo chromophore. Full-scale tests will be undertaken in which concentrated reactive dye effluent is dosed into a Bardenpho system. Decolorisation and degradation of reactive dyes have been

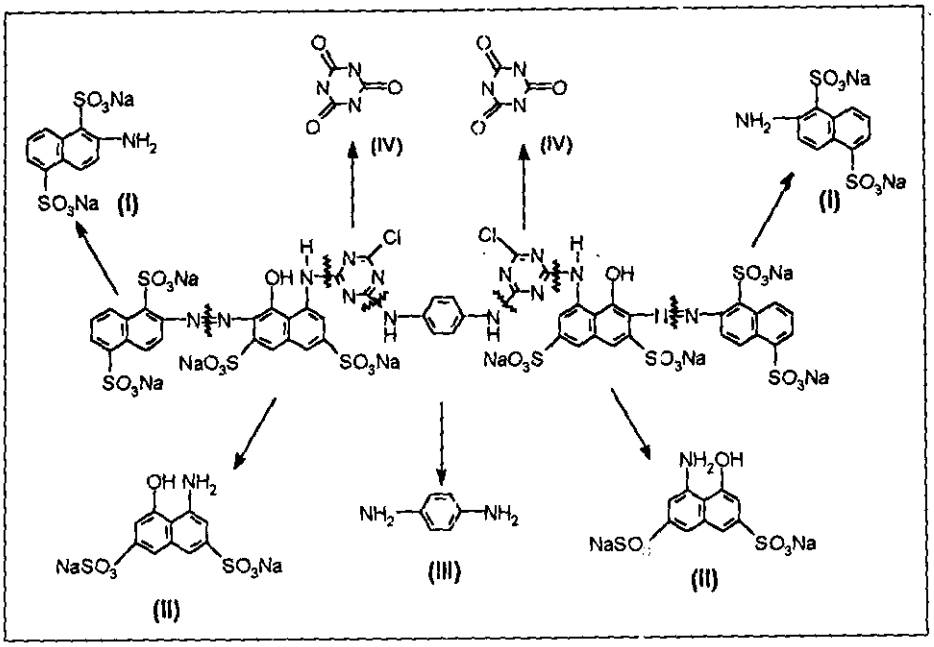

Figure 2
Proposed degradation pathway and products of C.I. Reactive Red 141

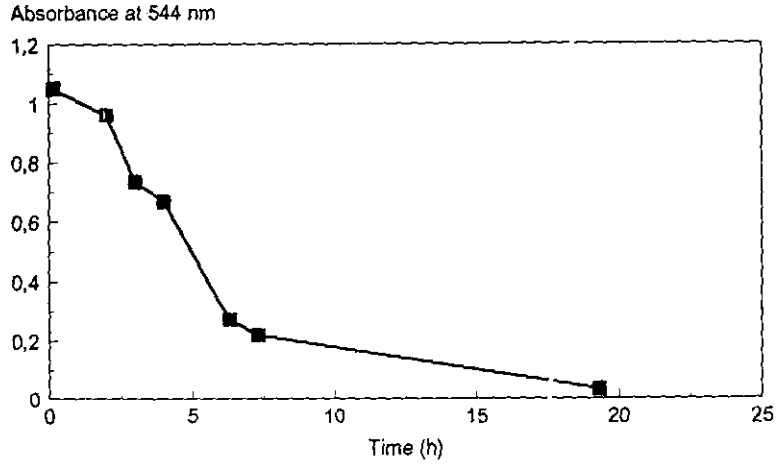

Figure 3
Rate of decolorisation of C.I. Reactive Red 141 in biomass from the 5-stage Bardenpho reactor

achieved in laboratory anaerobic systems. A second full-scale trial will be undertaken in which concentrated reactive dye effluent together with other concentrated organic rich textile effluents will be added to a primary sewage sludge digester. A possible route for the disposal of textile concentrates would therefore be to transport them to a sewage treatment works and dose them into the primary sludge anaerobic digesters or the anoxic zone of a nutrient removal activated sludge process.

Acknowledgements

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References

BROWN, D and LABOUREUR, P (1983) The degradation of dyestuffs Part I: Primary biodegradation under anaerobic conditions. *Chemosphere* **12** 397-404.

CARLIELL, C (1993) Biological Degradation of Azo Dyes in an Anaerobic System. M.Sc. Eng. Thesis, Department of Chemical Engineering, University of Natal, Durban.

DE HAAS, DW, BORAIN, GP and KERDACHI, DA (1993) Review of treatment performance at Hammarsdale Waste-water Works with special reference to alum dosing. *Water SA* **19**(2) 93-106.

ENDS REPORT (1993) Textile Industry Threads its Way Through the Effluent Challenge. No. 225, Industry Report No. 58: 15-18.

HARMER, C and BISHOP, P (1992) Transformation of azo dye AO-7 by wastewater biofilms. *Water Sci. Technol.* **26** 627-636.

KREMER, FV (1989) Anaerobic Degradation of Monoazo Dyes. Ph.D. Thesis, Department of Civil and Environmental Engineering, University of Cincinnati.

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