PERGAMON

Chemosphere 44 (2001) 1169–1176

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Azo dye decolourisation by anaerobic granular sludge

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Received 8 March 2000; accepted 23 June 2000

Abstract

The decolourisation of 20 selected azo dyes by granular sludge from an upward-flow anaerobic sludge bed (UASB) reactor was assayed. Complete reduction was found for all azo dyes tested, generally yielding colourless products. The reactions followed first-order kinetics and reaction rates varied greatly between dyes: half-life times ranged from 1 to about 100 h. The slowest reaction rates were found for reactive dyes with a triazine reactive group. There was no correlation between a dye's half-life time and its molecular weight, indicating that cell penetration was probably not an important factor. Since granular sludge contains sulphide, eight dyes were also monitored for direct chemical decolourisation by sulphide. All these dyes were reduced chemically albeit at slower rates than in the presence of sludge at comparable sulphide levels. Increasing sulphide concentrations, even when present in huge excess, stimulated the azo reduction rate. The results indicate that granular sludge can decolourise a broad spectrum of azo dye structures due to non-specific extracellular reactions. Reducing agents (e.g., sulphide) in sludge play an important role. The presence of anaerobic biomass is probably beneficial for maintaining the pools of these reduced compounds.

Keywords: Biological reduction; Chemical reduction; Reduction rates; Sulphide; Autoxidation

1. Introduction

One of the main problems associated with the treatment of textile wastewater is the removal of dyes. Most (60–70%) of the more than 10,000 dyes applied in textile processing industries are azo compounds, i.e., molecules with one or more azo (N = N) bridges linking substituted aromatic structures (Carliell et al., 1995). Discharge of azo dyes is undesirable not only for aesthetic reasons but also because many azo dyes and their breakdown products are toxic to aquatic life (Chung and Stevens, 1993) and mutagenic to humans (Chung et al., 1992).

Azo dyes are resistant to biodegradation under aerobic conditions (Pagga and Brown, 1986; Jimenez et al., 1988; Shaul et al., 1991; Ganesh et al., 1994; Pagga, 1994), but they undergo reductive fission of the azo linkage relatively easily under anaerobic conditions (Brown, 1981; Brown and Laboureur, 1983; Carliell et al., 1994; Razo-Flores et al., 1997; Beydilli et al., 1998). Although the phenomenon of anaerobic azo reduction is unanimously accepted, some aspects of the reaction mechanism remain to be clarified. Different observations have been reported on the involvement of enzymes and the location of the reaction and its kinetic order.

High-rate anaerobic treatment systems have been considered for the treatment of azo dyes in textile industry wastewater (Seshadri et al., 1994; FitzGerald and Bishop, 1995; An et al., 1996; Donlon et al., 1997). However, due to the wide variety of dyes used in the industry, a broad capability of the biomass in these reactor systems to reduce different dye structures needs to

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0045-6535/01/$ - see front matter
PII: S0045-6535(00)00270-8

be ascertained. The goal of this research was to evaluate the feasibility of granular sludge in upward-flow anaerobic sludge bed (UASB) reactors to reduce 20 different types of azo dyes. Since sludge granules contain high concentrations of chemically reactive sulphide, both the biological and chemical activity of the sludge towards azo reduction was considered.

2. Materials and methods

The biological dye decolourisation assays were conducted in 120 ml serum bottles containing 50 ml of medium and an overlying headspace composed of N_2/CO_2 (80%/20%) which was sealed with a butyl rubber stopper. The primary electron donating substrate of the medium was composed of 2 g l^{-1} chemical oxygen demand (COD) of an NaOH-neutralised volatile fatty acids (VFA) mixture containing acetate, propionate and butyrate in a COD-based ratio of 1:10:10. The basal nutrients of the medium were composed of 2.8 g NH_4Cl l^{-1}, 0.057 g $CaCl_2$ l^{-1}, 2.5 g KH_2PO_4 l^{-1} and 1 g $MgSO_4 \cdot 7H_2O$ l^{-1} and the medium was buffered at a pH of 7.3 ± 0.2 with $NaHCO_3$ (5 g l^{-1}). Non-adapted anaerobic granular sludge was added to the medium at a concentration of 1.5 g l^{-1} volatile suspended solids (VSS). The medium was flushed with the N_2/CO_2 (80%/20%) and preincubated with the sludge for 2–3 days. The background level of sulphide in the medium was 0.7 ± 0.02 mM. The selected dye was added to a final concentration of approximately 0.3 mM (100–300 mg l^{-1}) with a syringe from a concentrated stock solution. The serum bottles were incubated at 30°C in a rotary shaker at 50 rpm. At selected intervals, colour was measured spectrophotometrically at the dye's wavelength of maximum absorbance (λ_{max}). For this purpose, samples were centrifuged after dilution to less than 1 absorbance unit (AU) in a phosphate buffer (10.86 g l^{-1} $NaH_2PO_4 \cdot 2H_2O$; 5.38 g l^{-1} $Na_2HPO_4 \cdot H_2O$) that contained ascorbic acid (200 mg l^{-1}) to effectively prevent autoxidation. The background light absorbance of the control medium in the buffer was less than 0.5% of the absorbance due to dye containing medium in the buffer and could therefore be neglected.

The chemical decolourisation assays were conducted identically as the biological assays with the exception

Table 1
Overall results of azo dye decolourisation by anaerobic granular sludge

Dye	Purity (%)	λ_{max} (nm)	Decolourisation ($\%_{max}$)	k (d^{-1})[a]
Acid orange 7	98	484	99	1.49 ± 0.07
Acid red 266	NA[b]	492	95	0.20 ± 0.07
Acid yellow 137	NA	456	95	0.35
Acid yellow 159	NA	362	97	0.72
Basic red 23	NA	526	99	10
Direct black 19	NA	675	99[c]	3 ± 1[d]
Direct black 22	NA	484	99[c]	NM[e]
Direct blue 53	85	608	99	0.24
Direct blue 71	50	579	100	0.61 ± 0.04
Direct red 79	NA	510	97	16.6 ± 1.6
Direct red 81	50	509	99	7.8 ± 0.3
Direct yellow 4	70	402	95	1.03 ± 0.05
Direct yellow 12	65	401	86	1.17 ± 0.07
Direct yellow 50	60	402	99	2.0 ± 0.3
Mordant orange 1	80	373	97	1.74 ± 0.07
Mordant yellow 10	85	355	95	1.86 ± 0.05
Reactive black 5	55	595	99	5.0 ± 0.9
Reactive orange 14	NA	433	98	0.17 ± 0.01
Reactive orange 16	50	492	97	2.1 ± 0.4
Reactive red 2	50	539	100[f]	0.31 ± 0.03
Reactive red 4	50	521	99[f]	0.45 ± 0.02
Reactive yellow 2	50	405	73	0.01

[a] k-values (first-order rate constants) were obtained from fitting Eq. (1) to the complete decolourisation curve (monoazo dyes) or to the first part of the decolourisation curve (disazo and polyazo dyes); for experiments which were replicated, standard deviations are mentioned behind the ± sign.
[b] NA = information not available.
[c] Dye does not dissolve well: the decolourisation of the water phase is possibly a combination of reduction, adsorption and precipitation.
[d] Dye does not dissolve well: very rough estimate of k.
[e] Dye does not dissolve well: rate could not be measured.
[f] Reaction products are yellow.

that the granular sludge and VFA were excluded from the medium and sulphide was added to final concentrations ranging from 1 to 70 mM. The colour was measured as light absorbance at selected time intervals at each dye's λ_{max} as described previously for the biological assay.

To assess autoxidation of the aromatic amines formed during dye reduction, samples of completely decolourised dye solutions were brought into 1.5 ml microcentrifuge tubes which were left open to the air for 5–10 min, 1 h and 1 day, respectively, prior to dilution in phosphate buffer without ascorbic acid. After centrifugation, the 200–800 nm colour spectrums were scanned and compared with scans of original dye solutions in phosphate buffer.

The dyes were purchased from either Aldrich (Gillingham, England), Acros (Geel, Belgium), Sigma (Bornem, Belgium), Sigma–Aldrich (Steinheim, Germany), Crompton & Knowles (Tertre, Belgium) or Ciba-Geigy (Basel, Switzerland) and used without any further purification. As far as available, the purities of the dyes according to the manufacturer are mentioned in Table 1 and the structure formulas are shown in Figs. 1(a) and (b) (colour index generic names are used). For acid yellow 137, acid yellow 159 and basic red 23, the structure formulas are not known.

Anaerobic granular sludge came from an alcohol distillery (NEDALCO, Bergen op Zoom, The Netherlands).

3. Results

3.1. Biological azo dye reduction

The decolourisation of 20 azo dyes by anaerobic granular sludge was measured as the decrease of visible light absorbance at the previously assessed wavelength

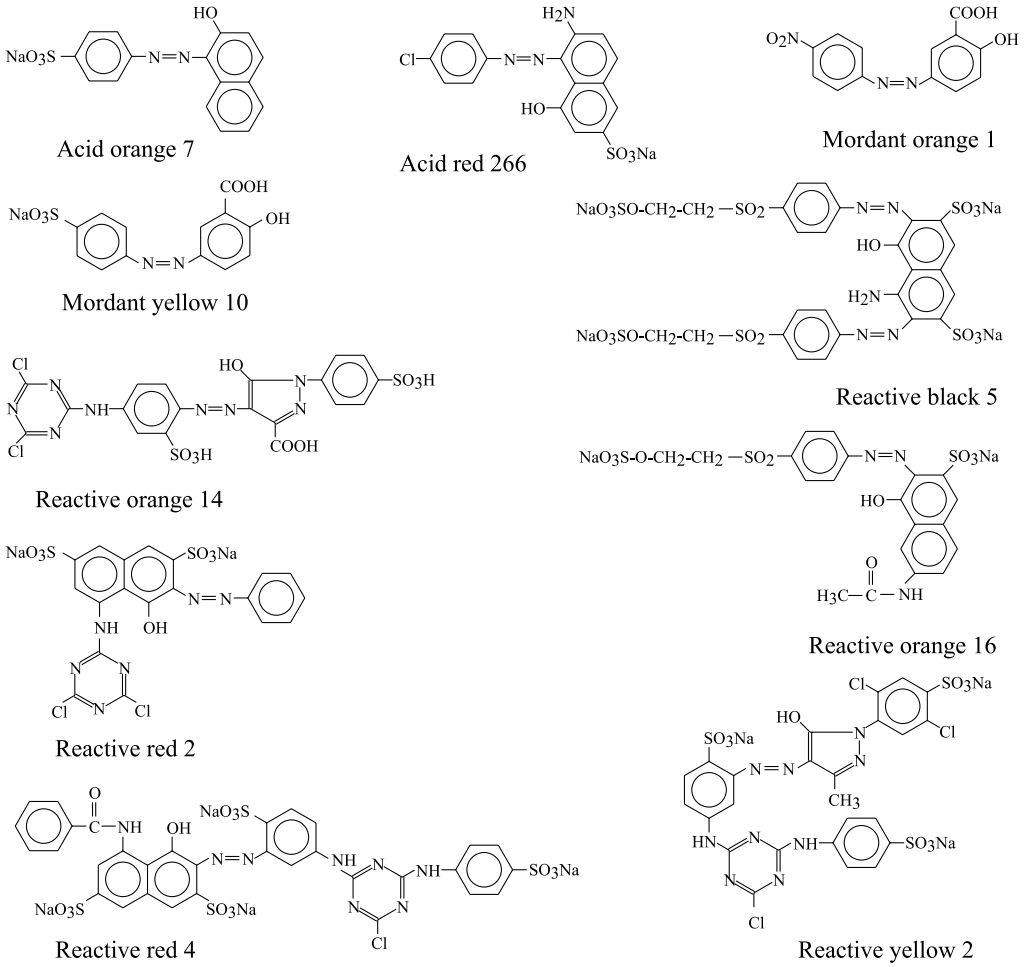

Fig. 1. (a) Structural formulas of the acid, mordant and reactive dyes used in this study; (b) structural formulas of the direct dyes used in this study.

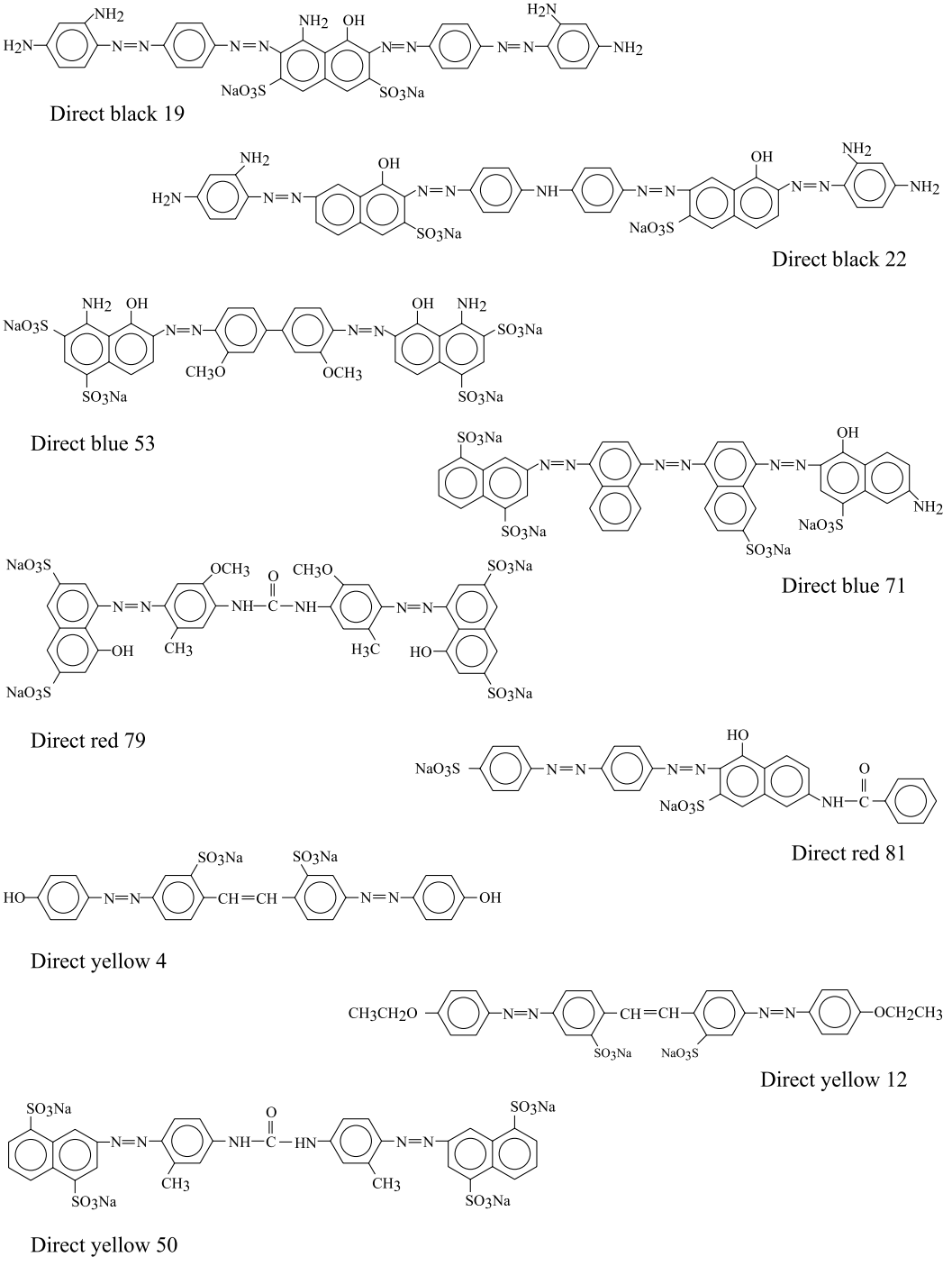

Fig. 1. (continued)

of maximum absorbance (λ_{max}). As summarised in Table 1, all azo dyes studied were found to be decolourised. The reactions proceeded without lag phase. The decolourisation was complete or nearly complete (>95% decrease of absorbance at λ_{max}) for most of the dyes. Important exceptions were direct yellow 12 and reactive yellow 2. Direct yellow 12 formed a new absorption peak with a maximum at 336 nm, close to λ_{max}, resulting in relatively high (~14%) residual absorbance at the λ_{max}. reactive yellow 2 had an exceptionally slow rate of

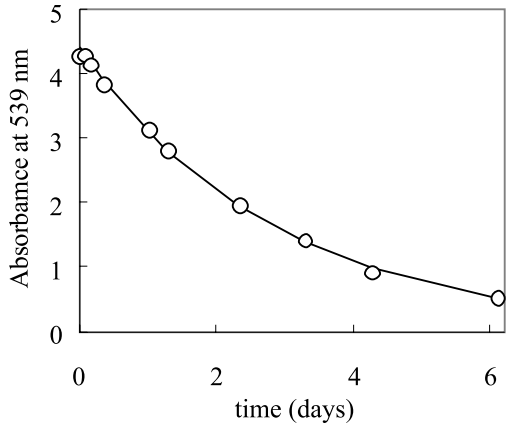

Fig. 2. Decolourisation of reactive red 2 in the presence of anaerobic granular sludge measured values (○) and first-order fit (–).

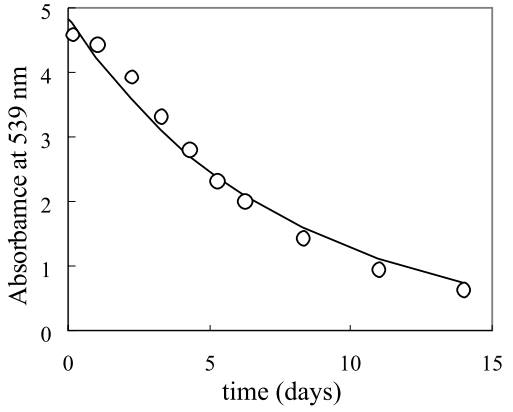

Fig. 3. Decolourisation of reactive red 2 by sulphide (initial sulphide concentration 2.8 mM) measured values (○) and first-order fit (–).

decolourisation, which was not yet complete after 342 days of incubation.

In most cases, the reaction products were colourless. Two exceptions were reactive red 2 and reactive red 4, in which a shift from red to yellow was observed. The decolourisation of the azo dyes in all cases proceeded without a lag phase. In the cases of monoazo dyes, the reaction followed first-order kinetics as shown for the example of reactive red 2 in Fig. 2. In contrast, dyes with more than one azo linkage displayed multiphase kinetics.

The first-order rate constants (k) resulting from fitting Eq. (1) to the whole curve (monoazo dyes) or to the first part of the curve (disazo and polyazo dyes) are listed in Table 1.

$$A_t = A_0 e^{-kt}, \quad (1)$$

where A_t is the light absorbance at λ_{max} at a given time (t); A_0 the light absorbance at λ_{max} at time 0; k the first-order rate constant; and t is the time.

Under the applied conditions, k-values varied greatly between different dyes yielding half-life times between 1 and 100 h. No correlation between k and molecular weight could be observed. For instance, the large dye direct red 79 ($M_W = 1049$ g mole^{-1}) decolourised much faster than the small dye mordant orange 1 ($M_W = 287$ g mole^{-1}). However, the four dyes containing triazine as a reactive group (reactive orange 14, reactive red 2, reactive red 4, reactive yellow 2) were among the dyes which were reduced at the slowest rates.

3.2. Chemical azo dye reduction

Since anaerobic granular sludge contains inorganic reducing agents like sulphide, direct chemical azo reduction by sulphide was investigated. All eight dyes tested (acid orange 7, direct black 19, direct red 81, direct yellow 4, direct yellow 50, mordant orange 1, mordant yellow 10 and reactive red 2) were found to be reduced by sulphide. In contrast to what was found for biological azo reduction, the decolourisation curves of most of these dyes deviated slightly from first-order kinetics. As a result of catalysis by azo reduction reaction products (van der Zee et al., 2001), a lag phase was observed immediately after dye addition. Thereafter, dye decolourisation accelerated in time before assuming typical first-order kinetics (see Fig. 3). This effect was especially evident at low dye or sulphide concentrations. Due to this deviation, the k-values obtained with data fitted with first-order kinetics were considered as pseudo first-order rate constants.

At comparable sulphide concentrations, azo reduction rates were stimulated by the presence of sludge. For example, the pseudo first-order rate constant (k) for the reduction of reactive red 2 by 1.3 mM of sulphide was 0.06 d^{-1}which was considerably lower than the k in the presence of anaerobic granular sludge (1.5 g VSS l^{-1}) of 0.3 d^{-1} at an initial sulphide concentration of 0.8 mM.

The pseudo first-order rate constants of dye reduction rates (k-values) increased with increasing sulphide concentration (Fig. 4). Up to a sulphide concentration of 0 to 60–70 mM, a more or less linear relationship between k and sulphide concentration was observed for reactive red 2 and acid orange 7 (a slow decolourising and a moderately slow decolourising dye, respectively). In contrast, for the fast decolourising direct red 81, the increase of k declined at high sulphide concentrations.

3.3. Autoxidation

The products of anaerobic azo cleavage are aromatic amines, which have been reported to undergo autoxi-

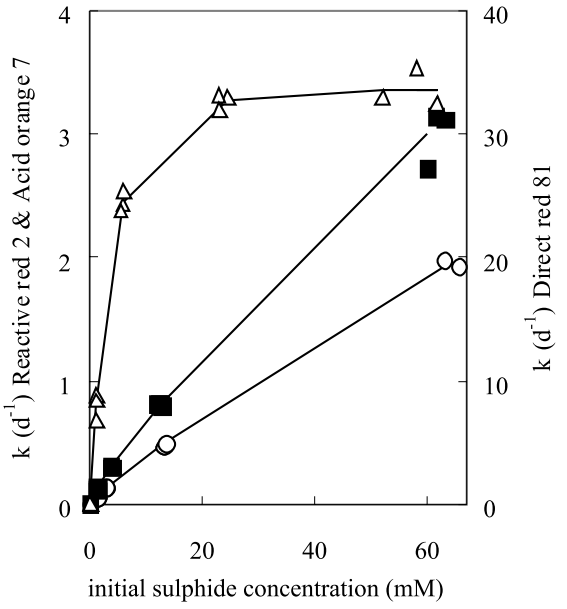

Fig. 4. Effect of sulphide on the chemical decolourisation of reactive red 2 (○), acid orange 7 (■) and direct red 81 (△).

dation reactions when they are exposed to oxygen (Noertemann et al., 1994; Kudlich et al., 1999). Therefore, samples from decolourised dye solutions of the biological assays were exposed to air to investigate this phenomenon qualitatively. With the exceptions of direct yellow 12, direct yellow 50 and reactive yellow 2, all decolourised solutions of azo dyes formed autoxidised coloured products upon exposure to oxygen. Generally, the autoxidation reactions proceeded quickly with colour development after only a few minutes of exposure to air, which was also evident from a largely altered UV-VIS spectrum. However, prolonged exposure to air generally did not result in further changes of the UV-VIS spectrum. Only in two cases (mordant orange 1 and acid orange 7) did autoxidation lead to the formation of clearly visible flocs, which could be separated by centrifugation.

4. Discussion

The results of this study indicate that granular sludge from high-rate anaerobic bioreactors can reduce and decolourise a broad spectrum of azo dye structures without any apparent lag phase. The rate of decolourisation is not dependent on the molecular weight of the dye indicating that cell permeation is probably not an important issue in the reductive mechanism. This observation combined with the non-specificity and lack of any lag-phase points to a non-enzymatic extracellular reaction mechanism involving reduced compounds. The mechanism is supported by the observation that sulphide, which is abundantly present in sludge, can directly cause the chemical reduction of azo dyes. Furthermore, data in the literature also suggest the involvement of reduced compounds causing direct chemical reduction of azo bonds, such as zero-valent iron (Weber, 1996; Cao et al., 1999) as well as reduced biochemical cofactors, including reduced flavins (Gingell and Walker, 1971) and NADH (Nam and Renganathan, 2000). Since azo dyes could be decolourised by sulphide, biological activity is not a prerequisite for azo reduction. As sulphide is inevitably present in anaerobic sludge environments, chemical azo reduction will contribute to the overall decolourisation process under 'living' anaerobic conditions. Nevertheless, at comparable sulphide concentrations, azo reduction proceeds faster in the presence of sludge. The exact nature of the presence of sludge and living organisms in contributing to an accelerated dye decolourisation rate is not fully known. An important plausible role of 'living bacteria' in the sludge could be the regeneration of reducing agents, such as sulphide, ferrous iron and reduced biochemical cofactors. Also, organic matter in the sludge may contain humic substances, which are known to accelerate reductive processes by redox mediation. The chemical reduction of particle bound 4-aminoazobenzene by zero valent iron was accelerated by the quinone, juglone (Weber, 1996). Also, the reduction of amaranth (an azo dye) by bacteria was accelerated by the presence of another quinone, anthraquinone sulphonate (Kudlich et al., 1997).

The course of the decolourisation process approximates first-order kinetics with respect to dye concentration. First-order kinetics with respect to dye concentration have also been reported by Wuhrmann et al. (1980), Weber and Wolfe (1987), Weber (1991) and Carliell et al. (1994), whereas other researchers found zero-order kinetics (Dubin and Wright, 1975; Brown, 1981; Harmer and Bishop, 1992). A probable explanation for these contradictory observations is that the rate-limiting step in the reduction of azo dyes may differ between the different experimental conditions studied. In pure cultures, for instance, the production of reducing equivalents, a zero-order process (Dubin and Wright, 1975), is far more likely to be rate-determining than in anaerobic sludge environments, where reducing equivalents are abundantly present. In the latter case, it can be assumed that the transfer, rather than the production, of reducing equivalents is rate-determining, which is supported by the observation that increasing sulphide concentrations speeded up the azo reduction rate even up to very high concentrations.

The ability of granular sludge to reduce a broad spectrum of dyes holds promise for the application of

high rate anaerobic systems as a feasible first stage in the complete removal of azo dyes from wastewater. However, the kinetic data predict that reactive dyes with a triazine reactive group are reduced slowly. Long residence times would be necessary to reach a satisfying extent of decolourisation. However, this problem may be overcome, as the results presented here reveal shortage of reducing equivalents, and literature data indicate that redox mediators can be used to accelerate the transfer of reducing equivalents.

During aerobic post-treatment of anaerobically treated, azo dye containing wastewater, there will be competition between biodegradation and autoxidation of aromatic amines. The autoxidation of aromatic amines in a subsequent aerobic post-treatment step may be problematic, not only because the formed products are coloured but also because some of these compounds, e.g., azoxy compounds, may cause toxicity (Field et al., 1995). It may as well be possible, however, that autoxidation leads to the formation of large, bulky, non-toxic, 'humic' polymers which can easily be separated from the water phase.

Acknowledgements

The authors gratefully acknowledge the financial support from the Dutch governmental Economy Ecology Technology (EET) programme 97007 'Closed loop water cycling in textile processing industries'.

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