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Effect of different redox mediators during thermophilic azo dye reduction by anaerobic granular sludge and comparative study between mesophilic (30 °C) and thermophilic (55 °C) treatments for decolourisation of textile wastewaters

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Abstract

The impact of different redox mediators on colour removal of azo dye model compounds and textile wastewater by thermophilic anaerobic granular sludge (55 °C) was investigated in batch assays. Additionally, a comparative study between mesophilic (30 °C) and thermophilic (55 °C) colour removal was performed with textile wastewater, either in the presence or absence of a redox mediator. The present work clearly evidences the advantage of colour removal at 55 °C compared with 30 °C when dealing with azo coloured wastewaters. The impact of the redox mediators anthraquinone-2,6-disulfonate (AQDS), anthraquinone-2-sulfonate (AQS) and riboflavin was evident with all dyes, increasing decolourisation rates up to 8-fold compared with the mediator-free incubations. The generation of the hydroquinone form AH_2QDS, i.e. the reduced form of AQDS, was extremely accelerated at 55 °C compared with 30 °C. Furthermore, no lag-phase was observed at 55 °C. Based on the present results we postulate that the production/transfer of reducing equivalents was the process rate-limiting step, which was accelerated by the temperature increase. It is conclusively stated that 55 °C is a more effective temperature for azo dye reduction than 30 °C, which on the one hand can be attributed to the faster production/transfer of reducing equivalents, but also to the decrease in activation energy requirements.

Keywords: Azo dye; Colour removal; Thermophilic; Mesophilic; Anaerobic; Redox mediator; Textile wastewater

1. Introduction

Dyes make the world more beautiful through coloured substances, but on the other hand they represent a serious pollution problem for the environment. Almost one million tons of dyes are annually produced in the

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world, of which azo dyes, characterized by an azo-bond (R_1—N=N—R_2), represent about 70% by weight (Hao et al., 2000). Azo dyes are the most common synthetic colourants released to the environment via textile, pharmaceutical and chemical industries (Chung et al., 1992). The discharge of azo dyes in water bodies is problematic not only for aesthetic reasons, but also because azo dyes and their cleavage products (aromatic amines) are carcinogenic (Brown and DeVito, 1993; Weisburger, 2002).

The biological treatment of azo dye wastewaters has been extensively researched. Under aerobic conditions low colour removal efficiencies are achieved because oxygen is a more efficient electron acceptor, therefore having more preference for electrons than azo dyes (Stolz, 2001). In contrast, anaerobic treatment generally gives good colour removal efficiencies (Cervantes et al., 2001; Van der Zee et al., 2001; Dos Santos et al., 2003a). The anaerobic microorganisms not only generate the electrons to cleave the azo bond, but also maintain the low redox potential (<−50 mV) which is required for the transfer of reducing equivalents to the dye molecule (Beydilli et al., 1998; Bromley-Challenor et al., 2000). Although a complete mineralization cannot generally be reached anaerobically, the reductive transformation increases the susceptibility of the aromatic molecule to oxygenases attack (Field et al., 1995). Therefore, for most of the azo dyes, a sequenced anaerobic/aerobic treatment is required not only for the reduction of the azo dye but also the mineralization of its cleavage products (Tan et al., 2000).

The transfer of reducing equivalents from a primary electron donor (co-substrate) to a terminal electron acceptor (azo dye) generally acts as the process rate-limiting step in anaerobic azo dye reduction (Van der Zee et al., 2003). However, the addition of redox mediators has been shown to accelerate this electron transfer (Kudlich et al., 1997; Keck et al., 2002), and higher decolourisation rates can be achieved in bioreactors operated with a low hydraulic retention time (HRT) (Cervantes et al., 2001; Dos Santos et al., 2003b).

Flavin-based compounds, such as flavin adenide dinucleotide (FAD), flavin adenide mononucleotide (FMN) and riboflavin as well as quinone-based compounds such as AQS, AQDS and lawsone have been extensively reported as redox mediators (Semdé et al., 1998; Cervantes et al., 2000; Rau et al., 2002). In this case, the mediator is biologically reduced by non-specific enzymes, as the direct electron acceptor of the primary electron donor. Secondly, the electrons are chemically transferred to the azo dye, the terminal electron acceptor, with consequent mediator regeneration (Fig. 1).

The impact of redox mediators on decolourisation rates has generally been investigated with azo model compounds, and their effectiveness in enhancing the decolourisation of textile wastewaters is still unclear due to the wide range of redox potentials among azo dyes (−180 to −430 mV) (Rau and Stolz, 2003). Moreover, a comparative study between mesophilic and thermophilic treatments of textile wastewater in the presence of a redox mediator has never been conducted. In the current investigation, the impact of different redox mediators upon colour removal of azo dye model compounds and textile wastewater was assessed in batch assays containing thermophilic anaerobic granular sludge (55 °C). Additionally, a comparative study between mesophilic and thermophilic decolourisation was performed using textile wastewater, either in the presence or absence of a redox mediator.

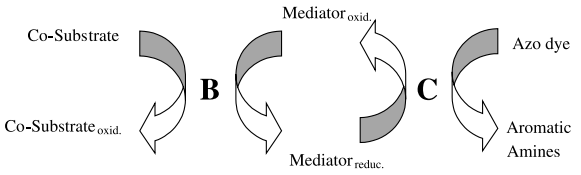

Fig. 1. Scheme of the biological and chemical steps involved in the azo dye reduction in the presence of redox mediators. Letters B and C are representing the biological enzymatic reaction and the pure chemical reaction, respectively.

2. Materials and methods

2.1. Chemicals

Reactive Red 2 (RR2), Acid Orange 7 (AO7) and Mordant Yellow 10 (MY10) were selected as azo dye model compounds (Fig. 2). Anthraquinone-2,6-disulfonate (AQDS), anthraquinone-2-sulfonate (AQS), riboflavin (vitamin B2) and cyanocobalamin (vitamin B12) were selected as redox mediator model compounds (Fig. 3).

Chemicals were purchased from Aldrich (Gillingham, UK), Sigma (Bornem, Belgium) or Acros (Geel, Belgium) and used without additional purification.

2.2. Seed inoculum and basal medium for decolourisation assays

2.2.1. Seed inoculum

Anaerobic granular sludge was collected from a full-scale mesophilic upflow anaerobic sludge blanket (UASB) reactor treating paper mill wastewater (Eerbeek, The Netherlands). The mesophilic sludge was acclimated for three months at 55 °C in an expanded granular sludge bed (EGSB) reactor (5.6 l) operating at a HRT of about 6 h and an organic loading rate (OLR) of 2.5 kg COD m^{-3} day^{-1}. The chemical oxygen demand (COD) consisted of a mixture of glucose and volatile

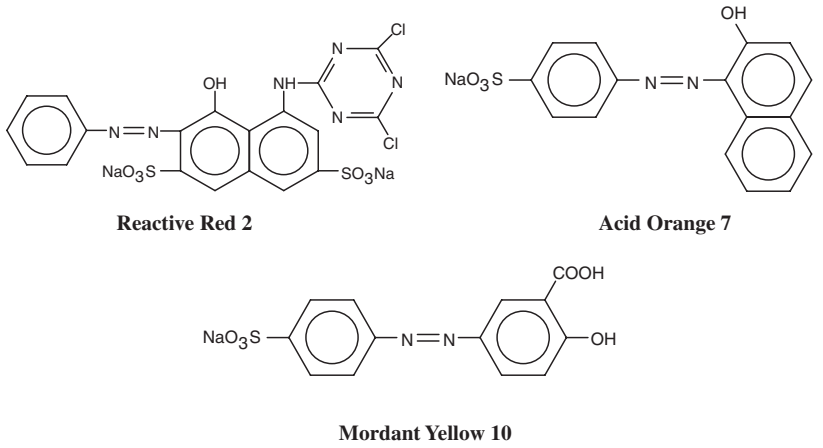

Fig. 2. Chemical structure of the azo dyes Reactive Red 2 (RR2), Acid Orange 7 (AO7) and Mordant Yellow 10 (MY10), used as model compounds.

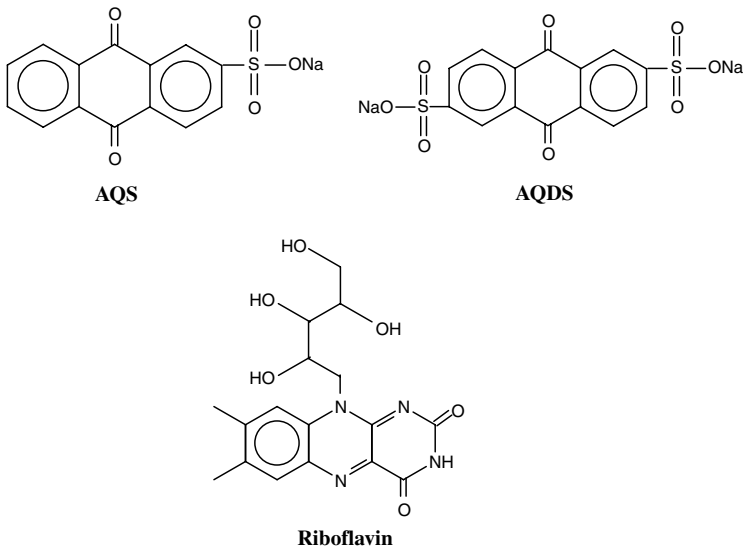

Fig. 3. Chemical structure of the external redox mediators anthraquinone-2-sulfonate (AQS), anthraquinone-2,6-disulfonate (AQDS) and riboflavin, used as model compounds.

fatty acids (VFA) at a COD ratio of 1:3. The neutralized VFA solution contained acetate, propionate and butyrate at a COD ratio of 1:1:1.

For batch tests at 30 °C the same mesophilic granular sludge was first acclimated to steady-state conditions in an EGSB reactor (30 °C) with the same co-substrate and hydraulic conditions beforehand reported.

2.2.2. Basal medium for activity tests

A previously described basal medium (Van der Zee et al., 2001) was used for tests with model compounds. It was buffered with 6.21 $g\,l^{-1}$ sodium bicarbonate at around pH 7.1. Resazurin was not included in the trace elements solution due to its mediating properties (Van der Zee et al., 2001).

The basal medium for the AQDS reducing capacity test contained ($mg\,l^{-1}$): NH_4Cl (100), K_2HPO_4 (50), $MgSO_4 \cdot 7H_2O$ (100) and $CaCl_2 \cdot 2H_2O$ (5), 1 $ml\,l^{-1}$ trace elements and 1 $ml\,l^{-1}$ vitamins solution. The medium was buffered with 6.21 $g\,l^{-1}$ sodium bicarbonate at around pH 7.1. The vitamin solution contained ($mg\,l^{-1}$): biotin (20), *p*-aminobenzoate (50), pantothenate (50), folic acid dihydrate (20), lipoic acid (50), pyridoxine (100) and nicotinamide (50).

When a wastewater derived from cotton processing textile factory was investigated, it was used undiluted and without the addition of nutrients or trace elements. The pH was adjusted to 7 with NaOH or HCl.

2.2.3. Activity test

Inoculation took place by adding 1.3 ± 0.1 g volatile suspended solids (VSS) l^{-1} of the previously described stabilized sludge to 117-ml serum bottles with 50 ml basal medium and sealed with butyl rubber stoppers. Anaerobic conditions were established by flushing the headspace with N_2/CO_2 (70%:30%) and 2 g COD l^{-1} (glucose:VFA mixture at a COD ratio of 1:3) co-substrate was added as electron donor and carbon source. After a pre-incubation time of two days, the azo dyes (variable) and redox mediators (variable) were added. Sterile controls were autoclaved once at 122 °C for 240 min and again following a five days incubation period, after which sterile co-substrate, mediator and dye stock solutions were added. The pH and the amount of VSS were determined upon completion of the experiment.

2.2.4. Effect of different redox mediators on thermophilic azo dye reduction at 55 °C

2.2.4.1. Model compounds. The impact of the redox mediators AQDS, AQS, riboflavin and cyanocobalamin (vitamin B12) on the thermophilic anaerobic reductive transformation of three azo dyes was investigated. The azo dyes (0.3 mM) were incubated together with co-substrate (2 g COD l^{-1}) either in the presence or absence of mediator (0.012 mM). Autoclaved sludge was used as a control for dye adsorption to the sludge and abiotic azo dye reduction by reducing agents. A sludge-free control was used to monitor the azo dye stability at 55 °C.

2.2.4.2. Decolourisation of acid dye wastewater. The three most efficient redox mediators obtained from the previous study were incubated at 55 °C with acid dye wastewater from a silk yarn-processing factory in Italy. The mediator concentration was increased from 0.012 to 0.024 mM to assess its impact on the decolourisation rates. An excess co-substrate concentration of 2 g COD l^{-1} (glucose:VFA mixture at a COD ratio of 1:3) was used to avoid any lack of primary electron donor. The previously stabilized thermophilic sludge was taken from a 4 °C storage and incubated with the wastewater. Abiotic colour removal was assessed with both autoclaved sludge and sludge-free incubations.

2.2.5. Comparative study between mesophilic and thermophilic anaerobic treatments

2.2.5.1. Decolourisation of reactive dye wastewater. A comparative study between mesophilic (30 °C) and thermophilic (55 °C) conditions on anaerobic azo dye reduction was conducted with a reactive dyeing wastewater. The light brown wastewater was taken from a Belgian cotton processing textile factory. The best mediating compound from the previous screening was used at a concentration of 0.5 mM, either in presence or absence of 2 g COD l^{-1} of co-substrate, i.e. glucose:VFA mixture on a COD ratio of 1:3. Both previously stabilized mesophilic and thermophilic sludges were taken from 4 °C storage and incubated with the wastewaters.

2.2.5.2. AQDS as electron acceptor in mesophilic and thermophilic incubations with granular sludge. To compare the capacity of microbial communities to use AQDS as a terminal electron acceptor, an AQDS reducing capacity test (AH_2QDS) was performed. AQDS (1 mM) was added to basal medium incubated at both 30 and 55 °C, in which 2 g COD l^{-1} of a glucose:VFA mixture at a COD ratio of 1:3 was either present or absent. Sludge-free and autoclaved sludge bottles were used as controls for abiotic AQDS reduction. Both previously stabilized mesophilic and thermophilic sludges were taken from a 4 °C storage. The pH of the medium was 7 in all incubations.

2.2.6. Activation energy (E_a) determination during chemical decolourisation

The activation energy requirements of the model compound Reactive Red 2 (RR2, 0.3 mM) were determined in sludge-free incubations by way of chemical reaction between a reducing compound and the dye. Sulfide (4.5 mM) was selected as a reducing agent and incubated with RR2 and AQS (variable) at temperatures of 30, 45 and 55 °C. AQS (0.012 mM) was added to some of the bottles. The concentration of sulfide was measured initially and upon completion of the experiment.

2.3. Analysis

For azo dye model compounds, colour removal was determined photometrically (Spectronics 60, Milton-Roy Analytical Products Division, Belgium) according to Van der Zee et al. (2001), reading the absorbance at the maximum absorbance wavelength, i.e. RR2 at 539 nm, AO7 at 484 nm and MY10 at 355 nm. The extinction coefficients used (AU $cm^{-1}\,mM^{-1}$) were 33.3, 20.0 and 17.3 for RR2, AO7 and MY10, respectively.

Wastewater decolourisation was determined in a 1 cm quartz cuvette by scanning the VIS spectra (Perkin–Elmer UV/VIS Lambda 12, Rodgau–Jügesheim, Germany) and comparing the wavelengths of two absorbance peaks.

AH_2QDS was determined anaerobically in a Type B Coy anaerobic chamber (Coy Laboratory Products Inc., USA) under a N_2/H_2 (96%:4%) atmosphere, according to Cervantes et al. (2000). An extinction coefficient of 2.08 AU $mM^{-1}\,cm^{-1}$ at 450 nm was obtained by chemical reduction of AQDS in a hydrogen atmosphere, in the presence of a hydrogenation catalyst according to Kudlich et al. (1999).

Sulfide was determined photometrically by using the Dr. Lange cuvette method and VSS were analyzed according to APHA standard methods (1998).

3. Results

3.1. Effect of different redox mediators during thermophilic colour removal at 55 °C

3.1.1. Model compounds

Colour removal with living sludge in the presence of the azo dyes RR2 and AO7 followed a first-order reaction with respect to the dye concentration. However, for MY10 a zero-order reaction fit the colour data more accurately. Thus, the zero-order rate constant "k_0" (mM day^{-1}) and the first-order rate constant "k_1" (day^{-1}) were determined based on the dye concentration depletion.

The impact of the redox mediators AQDS, AQS and riboflavin at a catalytic concentration of 0.012 mM was evident with all three dyes, increasing the decolourisation rates up to 8-fold (Table 1) compared with the mediator-free bottles. Mediator addition did not affect the reaction-order. Riboflavin was by far the best mediator with the reactive azo dye RR2, increasing the k_1-value 3-fold compared with the AQS-supplemented incubation (Table 1). However, the same tendency was not observed with AO7 and MY10, for which AQS had a higher catalytic capacity than riboflavin.

Comparing the decolourisation rates between AQS- and AQDS-supplemented bottles, it is observed that the mediating capacity of AQS is much higher than AQDS, i.e. with RR2 1.8-fold and AO7 1.4-fold. Surprisingly, with MY10 all mediators catalyzed the colour removal at similar rates (Table 1).

Vitamin B12 was found to be a very poor redox mediator, having no effect on the decolourisation rates of AO7 and MY10 (Table 1). Based on this finding vitamin B12 was excluded during the investigations with textile wastewater.

Table 1
First-order constant (k_1) and zero-order constant (k_0) on colour removal of azo dye model compounds RR2, AO7 and MY10 by thermophilic (55 °C) anaerobic granular sludge, either in the presence or absence of the redox mediators AQS, AQDS, riboflavin and vitamin B12

Incubation	k_1/VSS (day^{-1} g^{-1} l)		k_0/VSS (day^{-1} g^{-1} mM l)
	RR2	AO7	MY10
Mediator-free	0.72	4.20	0.44
AQDS	1.07	5.38	0.64
Vitamin B12	0.87	3.83	0.36
Riboflavin	5.83	6.79	0.64
AQS	1.92	7.30	0.66

- 2 g COD l^{-1} co-substrate (glucose:VFA, at a COD ratio of 1:3).
- The first-order constant k_1 was calculated assuming a first-order reaction for RR2 and AO7. The zero-order constant k_0 was calculated assuming a zero-order reaction for MY10. The k-values were normalized by the amount of VSS present in the incubation (1.3 g VSS l^{-1}).
- The results are means of triplicate incubations.
- The concentration of azo dye and mediators are 0.3 and 0.012 mM, respectively.

3.1.2. Acid dyeing wastewater

All redox mediators accelerated the decolourisation rates of the acid dyeing wastewater, which can be observed from the spectra plotted in Fig. 4. The colour removal followed a first-order reaction both in the presence and absence of mediators (Fig. 5). However, the increase in decolourisation rate was not so evident as in the case of azo dye model compounds, regardless the higher concentration of mediator. For the mediator-supplemented incubations the k_1-value increased 1.3-fold for AQS, 1.2-fold for riboflavin and 1.1-fold for AQDS compared with the mediator-free incubation (Fig. 5).

3.2. Comparative study between mesophilic and thermophilic anaerobic treatments

3.2.1. Decolourisation of reactive dyeing wastewater

Batch assays showed that decolourisation under thermophilic conditions was distinctly faster than under mesophilic conditions (Fig. 6A and B). Moreover, the relative impact of the external redox mediator AQS (0.5 mM) on colour removal was considerably decreased under thermophilic conditions.

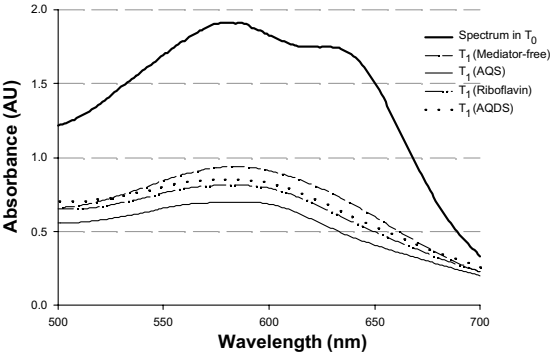

Fig. 4. Acid dyeing wastewater spectra after 22.5 h of incubation (T_1) in thermophilic (55 °C) incubations with granular sludge (1.3 g VSS l^{-1}). T_0 was the initial wastewater spectrum. Different redox mediators (0.024 mM) were added to some bottles and 2 g COD l^{-1} co-substrate. Sludge-free incubations were used to assess the chemical decolourisation, which contained wastewater, mediator and co-substrate. The results are means of duplicate incubations. The standard deviations were lower than 5% in all cases.

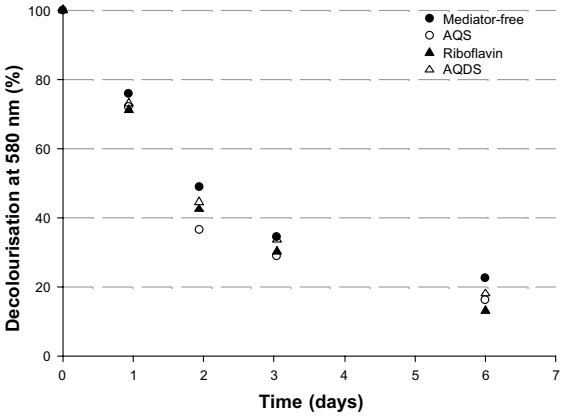

Fig. 5. Decolourisation at 580 nm of the acid dyeing wastewater by thermophilic (55 °C) granular sludge (1.3 g VSS l^{-1}). Different redox mediators (0.024 mM) were added to some bottles and 2 g COD l^{-1} co-substrate. The results are means of duplicate incubations. The standard deviations were lower than 5% in all cases.

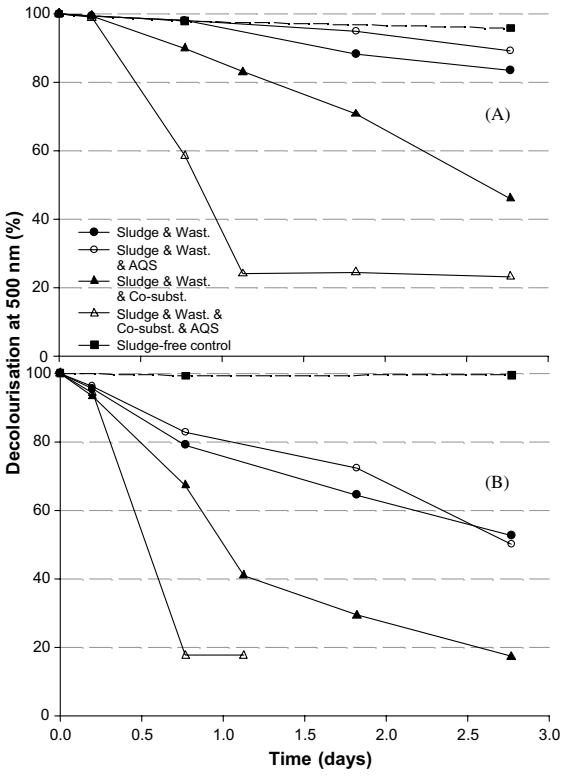

Fig. 6. Decolourisation of the textile wastewater at 500 nm by mesophilic (30 °C) (A) and thermophilic (55 °C) (B) granular sludge (1.3 g VSS l^{-1}). AQS (0.5 mM) was added to some bottles and 2 g COD l^{-1} co-substrate. The results are means of duplicate incubations. The standard deviations were lower than 5% in all cases.

Colour removal was accelerated by addition of co-substrate either in the presence or absence of mediator. Incubations supplemented with mediator in the absence of co-substrate showed no difference in rates (Fig. 6A and B). Thus, in the case of separate treatments of dyeing- and rinsing-step wastewaters the addition of co-substrate should be considered.

Negligible (<4%) colour removal occurred in sludge-free controls in the presence of AQS during the incubation time of 2.7 days.

3.2.2. AQDS as electron acceptor in mesophilic and thermophilic microbial incubations with granular sludge

Anaerobic granular sludge incubated under mesophilic and thermophilic conditions was capable to couple the reduction of AQDS as a final electron acceptor with the primary electron donor oxidation (co-substrate). The generation of AH_2QDS, the reduced form of AQDS, was greatly accelerated at 55 °C (Fig. 7) compared with 30 °C. Furthermore, no lag-phase was observed under thermophilic conditions. For instance, about 1 mM AQDS was completely reduced at 55 °C after 0.7 days of incubation, whereas mesophilic reduction after 0.7 days represented just 12.9% of this value (Fig. 7).

AH_2QDS formation in endogenous controls, autoclaved sludge and sludge-free incubations was negligible during the two day experiment at both 30 and 55 °C.

3.3. E_a determination during chemical decolourisation

The chemical decolourisation of RR2 by sulfide followed a first-order reaction with respect to the dye concentration. Thus, the first-order rate constant "k_1" (day^{-1}) was determined in each temperature tested. In

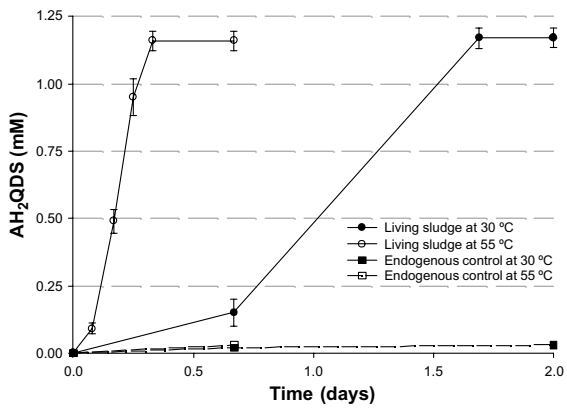

Fig. 7. AH_2QDS formation (1 mM) at both 30 and 55 °C temperatures. Measurements were conducted under anaerobic conditions, in which the samples were diluted in a bicarbonate buffer (60 mM, pH 6.8 ± 0.1), and the absorbance was read at 450 nm. The results are means of triplicate incubations.

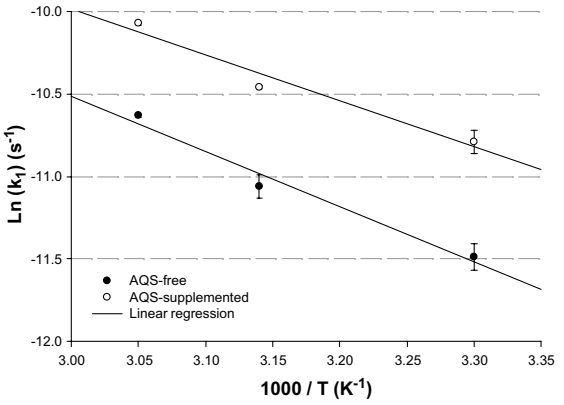

Fig. 8. Arrhenius plot and linear regression for the chemical decolourisation of RR2 (0.3 mM) by sulfide (4.5 mM) in sludge-free incubations. AQS (0.012 mM) was added to some of the bottles. The value of the ratio E_a/R was obtained by the slope of the linear regression, in which the parameter R was the universal gas constant (8.314 $J K^{-1} mol^{-1}$). The results are means of duplicate incubations with the vertical bars representing the standard deviations.

order to calculate E_a values $\ln(k_1)$ versus $1000/T$ was plotted, and the slope E_a/R was obtained by the linear regression. This ratio was multiplied by the universal gas constant R (8.314 $J K^{-1} mol^{-1}$) to obtain the E_a value. Fig. 8 shows that the Arrhenius equation could describe the chemical decolourisation of RR2 by sulfide at different temperatures. The slopes of the AQS-free and AQS-supplemented incubations were indeed different (Fig. 8). The calculated E_a values were 27.9 and 22.9 $kJ mol^{-1}$ for the AQS-free and AQS-supplemented incubations, respectively. Therefore, the activation energy was decreased 1.2-fold due to the addition of 0.012 mM-AQS.

4. Discussion

The present work clearly evidences the advantage of thermophilic treatment at 55 °C over mesophilic treatment at 30 °C for the decolourisation of azo coloured wastewaters (Fig. 1A and B). The normal rate limiting step, the transfer of reducing equivalents, was accelerated under thermophilic conditions. Both biotic and abiotic mechanisms may contribute to enhance the observed decolourisation under thermophilic conditions. The faster biological reduction of the redox mediator, AQDS, achieved by sludge incubations at 55 °C in comparison with 30 °C (Fig. 7), evidenced the biological contribution in enhancing the rate of electron transfer. On the other hand, AQS-supplemented incubations presented a lower E_a requirement during the chemical reduction of RR2 by sulfide (Fig. 8). Therefore, at 55 °C, the external mediator dosage can be decreased to achieve the colour removal requirements.

The improvement on the decolourisation rates at 55 °C due to the addition of low molecular weight redox mediators at catalytic concentration was clear with all tested azo dye model compounds (Table 1). Riboflavin and AQS had similar mediating properties, which is most likely related to the more or less equal redox potentials (E_0') of riboflavin and AQS (−208 and −225 mV, respectively) (Sober et al., 1970). However, riboflavin was a far better mediator than AQS with regard to the azo dye RR2 (Table 1). Such a result evidences that other factors, such as steric hindrance between azo dye and redox mediator, also determine the decolourisation rates in the system. This is in contrast with Chung et al. (1978), who tested different redox mediators in the reduction of tartrazine by *Bacteroides thetaiotaomicron*. The use of methyl viologen and benzyl viologen increased the decolourisation rates 4.5-fold. However, the E_0' of methyl viologen and benzyl viologen are −440 and −339 mV, respectively (Brown, 1981), although this high redox difference was not shown to affect decolourisation rates.

Walker and Ryan (1971) postulated that decolourisation rates are related to the electron density in the region of the azo bond. They suggested a colour removal increase by lowering the electron density in the azo link. Therefore, the use of redox mediators would not only tend to accelerate the reducing equivalents transfer to the terminal electron acceptor azo dye, but also to minimize the steric hindrance of the dye molecule (Bragger et al., 1997; Moir et al., 2001).

Vitamin B12 showed to be a very poor redox mediator with the azo dye model compounds (Table 1). This is probably due to its highly negative redox potential E_0' of −530 mV (Chiu and Reinhard, 1996), which in fact requires a higher reducing capacity than was available in the present anaerobic incubation. This is in accordance with Chiu and Reinhard (1996) who verified that vitamin B12 had little effect on carbon tetrachloride reduction in the absence of the external reductant cysteine. Therefore, the redox potential of the mediator E_0' is an indication of its catalytic capacity and must be in the range of −440 mV (Brown, 1981) and −50 mV (Rau and Stolz, 2003). Other information should be derived from the difference in steric and electro-chemical factors between mediator and azo dye.

In the acid dyeing wastewater experiment, an increase in decolourisation rates due to the addition of redox mediators at catalytic concentrations (0.024 mM) was not so evident (Figs. 4 and 5). This difference was probably due to the high dye COD concentration (1.4 $g l^{-1}$) and the properties of the dyes present in the wastewater. The dyebath contained three dyes, the final hue being a combination of red, yellow and blue. As the dyebath colour was dark blue, the blue dye was most

likely present in a greater concentration than the other two dyes. The structure of the dyes in the wastewater was not known, but many blue dyes have an anthraquinone structure. An experiment with anthraquinone dye Reactive Blue 5 showed that because this type of dye is electronically very stable, the chemical electron transfer from the reduced form of the mediator to the dye is less effective, being the rate-limiting step (data not shown, paper in progress).

Thermophilic anaerobic treatment showed a higher decolourisation rate compared with mesophilic anaerobic treatment (Fig. 6A and B) with the reactive dyeing wastewater. The impact of temperature on colour removal also corroborated Willets and Ashbolt (2000), who reported faster decolourisation rates at 55 °C compared to 35 °C while treating the azo dye Reactive Red 235 in UASB bioreactors free of external redox mediator. Furthermore, Laszlo (2000) reported a higher decolourisation of the azo dyes Orange II and Remazol Red F3B at 43 °C than at 28 °C by anaerobically incubating the facultative organism *Burkholderia cepacia* NRRL B-14803. Based on these reports and our present results we postulate that the transfer of reducing equivalents to the azo dye is the process rate-limiting step, which obviously is accelerated by the temperature increase. Thus, it is conclusively stated that 55 °C is a more effective temperature than 30 °C for colour removal, which brings good prospects on the application of thermophilic treatment for decolourisation processes.

At present, a comparative study between mesophilic (30 °C) and thermophilic (55 °C) anaerobic treatment of textile wastewater is being conducted in EGSB reactors, either in the presence or absence of redox mediator. The wastewater consists of a mix of water from the dyebath and the rinsing steps.

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