Bioresource Technology 101 (2010) 105–110

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Anthraquinone-2,6-disulfonate (AQDS) as a catalyst to enhance the reductive decolourisation of the azo dyes Reactive Red 2 and Congo Red under anaerobic conditions

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ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form 3 August 2009
Accepted 4 August 2009
Available online 29 August 2009

Keywords:
Anaerobic treatment
Congo Red
Reactive Red 2
Redox mediator
Co-substrates

ABSTRACT

In this study, we assessed the catalytic effect of anthraquinone-2,6-disulfonate (AQDS) to enhance the reductive decolourisation of the azo dyes Reactive Red 2 and Congo Red in batch and continuous-flow experiments. While testing the anaerobic sludge 1 in assays free of AQDS, the highest values for the first-order kinetic constant ($k1$) were found with co-substrates formate and glucose. In the assays that contained 50 μM of AQDS, the k1 values increased with all co-substrates tested, increasing by 3.5-fold when ethanol was the electron donor. The upflow anaerobic sludge blanket (UASB) reactors R1 (AQDS-free) and R2 (AQDS-supplemented) reached excellent decolourisation efficiencies (higher than 90%) even for the high Congo Red concentration tested (1.2 mM). However, electron donor depletion in the influent drastically decreased the colour removal capacity in both bioreactors. Reactor R2 presented higher stability and decolourisation efficiency compared to R1, indicating that the addition of a redox mediator can be valuable for treating dye-coloured wastewaters.

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1. Introduction

Azo dyes represent the most important class of dyes used in the textile industry. They are characterized by the presence of one or more azo groups (–N=N–) and the bonds between two or more aromatic rings (Zollinger, 1987).

The discharge of dye-coloured wastewaters in the environment represents both environmental and public health risks because of the negative ecotoxicological effects and bioaccumulation in wildlife (Chung and Stevens, 1993). Most importantly, many dyes and their reduced products are carcinogenic and mutagenic (Chung and Cerniglia, 1992). Additionally, colour in surface water is aesthetically undesirable and may affect photosynthesis, thereby compromising aquatic life (Manu and Chaudhari, 2002).

Methods currently used to treat textile wastewaters have technical and economical limitations (Dos Santos et al., 2007). Most of the physico-chemical methods that remove colour from waters are expensive, produce large amounts of sludge, and are inefficient for some soluble dyes. Biological treatment of dyed-waters can be cheaper than physico-chemical treatment, but some dyes are recalcitrant and toxic to microorganisms (Van der Zee and Villaverde, 2005).

Colour removal by aerobic bacteria is usually low (10–30%), because oxygen is a better electron acceptor compared to the azo dyes (Stolz, 2001; Dos Santos et al., 2007). However, under anaerobic conditions, azo dyes can act as electron acceptors, and higher decolourisation rates are usually reported (Manu and Chaudhari, 2002; Méndez-Paz et al., 2005; Dos Santos et al., 2005, 2007; Singh et al., 2007).

Redox mediators (RM) can enhance many reductive processes under anaerobic conditions, including azo dye reduction. Quinone-based compounds have shown to work as redox mediators by promoting electron transfer in chemical and microbiological reactions (Cervantes et al., 2001; Field and Brady, 2003; Dos Santos et al., 2007). Quinone-based compounds are enzymatically reduced by bacteria to form hydroquinones, which subsequently reduce azo dyes in a purely chemical reaction (Rau and Stolz, 2003), thereby increasing electron transfer from the electron donor, usually organic substrates, to the electron acceptor azo dye (Dos Santos et al., 2005).

The type and availability of electron donors are important in achieving good colour removal in bioreactors operated under anaerobic conditions (Van der Zee and Villaverde, 2005). Anaerobic microorganisms are capable of utilizing different electron donors to promote reductive decolourisation of dyes. However, each

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doi:10.1016/j.biortech.2009.08.015

anaerobic consortium has an optimal electron donor that gives the highest decolourisation rates.

Aromatic amines are formed during dye reduction and they are normally recalcitrant under anaerobic conditions. Since aromatic amines are toxic, carcinogenic, and contain Chemical Oxygen Demand (COD), post-treatment is needed (Field et al., 1995). Some investigations indicate that aromatic amines can be mineralized by aerobic bacteria, or by using chemical-oxidative processes (Pinheiro et al., 2004).

The present investigation aimed to evaluate, in batch and continuous-flow experiments, the catalytic effect of the compound AQDS to enhance the reductive decolourisation of the azo dyes Reactive Red 2 (RR2) and Congo Red (CR) in the presence of different co-substrates and operational conditions.

2. Methods

2.1. Materials

The azo dyes used in the experiments were RR2 (Procion Red MX-5B, ~50% of purity, Aldrich Chemical Company) and CR (analytical grade, Vetec), whose molecular structures are shown in Figs. 1 and 2. RR2 is a monoazo (MW of 615.34 g per mol) and CR is a diazo (MW of 696.98 g per mol). The compound anthraquinone-2,6-disulfonate (AQDS) (~98% of purity, Aldrich Chemical Company) was selected as a redox mediator, whose molecular structure is shown in Fig. 3. The compounds acetate, glucose, ethanol and formate were tested as co-substrates.

2.2. Batch experiments

Two anaerobic consortia were tested separately in batch experiments: consortium 1 (sludge 1) was collected from an UASB reactor placed in a brewery (Fortaleza, Ceará, Brazil); consortium 2

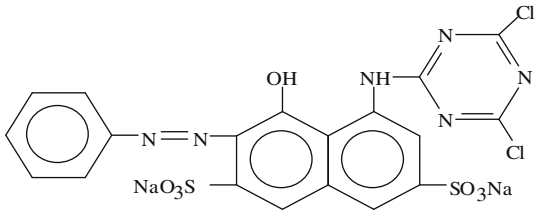

Fig. 1. Chemical structure of the azo dye Reactive Red 2.

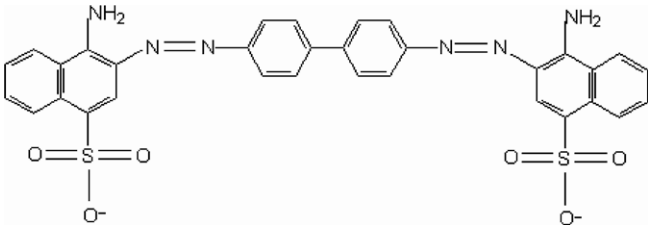

Fig. 2. Chemical structure of the azo dye Congo Red.

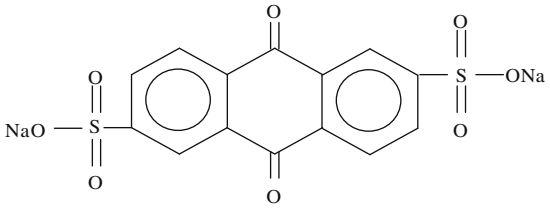

Fig. 3. Chemical structure of the redox mediator AQDS.

Table 1
Basal medium composition.

	Concentration (mg/L)
Macronutrients	
NH_4Cl	280
K_2HPO_4	250
$MgSO_4{\cdot}7H_2O$	100
$MgSO_4{\cdot}7H_2O$	10
Trace elements	
H_3BO_3	50
$FeCl_2{\cdot}4H_2O$	2000
$ZnCl_2$	50
$MnCl_2{\cdot}4H_2O$	500
$CuCl_2{\cdot}2H_2O$	38
$(NH4)_6Mo_7O_{24}{\cdot}4H_2O$	50
$AlCl_3{\cdot}6H_2O$	90
$CoCl_2{\cdot}6H_2O$	2000
$NiCl_2{\cdot}6H_2O$	92
$Na_2SeO_3{\cdot}5H_2O$	162
EDTA	1000
HCl	1*

* HCl concentration was 1 μL/L (36%).

(sludge 2) was collected from an UASB reactor placed in a paper-mill industry (Eerbeek, The Netherlands).

The anaerobic consortium was tested in the concentration of 1.5 g VSS (volatile suspended solids)/L. It was transferred to 117-mL batch assays, with 50 mL of basal medium that consisted of macronutrients and 1 mL/L of trace elements (concentrations are shown in Table 1). The medium was buffered with 2.5 g/L of sodium bicarbonate to keep the pH around 7.1.

Dyes and AQDS were tested with concentrations of 0.3 mM and 50 μM, respectively (Dos Santos et al., 2003). The co-substrates used in batch experiments were acetate, glucose, ethanol and formate, tested in the same concentration of 1.5 g COD/L.

Batch bottles were sealed with butyl rubber stoppers and aluminum crimp caps, and the headspace was purged with N_2:CO_2 (70%:30%) for 1 min to establish anaerobic conditions. Subsequently, organic substrates, dyes and redox mediators were added. The batch assays were placed in a room temperature of about 28 ± 2 °C under agitation of 150 rpm.

2.3. Continuous-flow experiments

Two UASB reactors were operated with a Hydraulic Retention Time (HRT) of 8 h, and kept at room temperature (28 ± 2 °C). The reactors were named R1 (AQDS-free) and R2 (AQDS-supplemented). Both reactors were 42.5 cm height and 4 cm in diameter, for a total volume of 0.53 L. Anaerobic consortium 1 was added to the reactors (30 g VSS/L), and the sludge blanket occupied approximately 2/3 of the reactor height.

The synthetic wastewater contained the same basal medium described for the batch experiments, the dye CR, cosubstrate and sodium bicarbonate (2.5 g/L). AQDS was added to R2 influent in the concentration of 50 μM. The influent was prepared 3 times a week and stored at 4 °C to avoid biological contamination.

The dye CR was tested in concentrations of 0.6 mM and 1.2 mM. Ethanol was selected as co-substrate and the initial organic loading rate (OLR) was 2.5 kg COD/m^3.day. Different OLRs were tested, and there was a period during which no co-substrate was added, in order to evaluate the consortium's capability to use the dye as a carbon and energy source.

2.4. Analyses and calculations

Colour removal of the RR2 and CR was determined photometrically at wavelength readings of 539 nm and 486 nm, respectively,

which gave the maximum absorbance for each dye. Samples were diluted in a phosphate buffer in Eppendorff tubes. In order to avoid turbidity interference, samples were centrifuged at 13,000 rpm for 2 min.

For batch experiments, sample collection was conducted visually observing colour loss in the bottles, at which samples from all bottles were usually collected.

For continuous-flow experiments, samples were collected from the influent and effluent of the reactors. Alkalinity, pH, volatile fatty acids (VFA) and chemical oxygen demand (COD) were analyzed twice a week, according to Standard Methods (APHA, 2005).

The first-order rate constant "$k1$" was determined for dye concentration using the Eq. (1):

$$A_t = A_0 \cdot e - kt \quad (1)$$

where, at is the absorbance at time "t", A_0 is the initial absorbance at $t = 0$, "k1" is the first-order rate constant (day^{-1}) and "t" is the time elapsed (days). Time was plotted against ln (A_t/A_0) and the k-value was estimated by the slope of a linear regression, using the Microsoft Excel solver tool.

3. Results and discussion

3.1. Effects of different electron donors on the reductive decolourisation of azo dyes by two anaerobic consortia

Table 2 shows the effect of different electron donors on RR2 reduction by two anaerobic consortia for AQDS-free assays. In terms of first-order kinetic constant, formate was the most effective electron donor to promote RR2 decolourisation with both consortia. However, compared to 97.8% found with glucose, the final colour removal efficiency with formate was only 75.6%, as shown in Table 2 and Fig. 4. Such behavior was likely due to the fact that formate was preferentially converted into methane, suggesting a competition between dye reduction and methanogenesis (Dos Santos et al., 2006).

Méndez-Paz et al. (2005), investigating colour removal of the azo dye Acid Orange 7 in batch assays supplemented and deprived with co-substrate, showed that dye reduction was considerably enhanced when glucose was added as co-substrate. Dos Santos et al. (2005) tested glucose as the electron donor in the same concentration as used in the present investigation, and obtained 100% RR2 (0.3 mM) reduction with mesophilic and thermophilic granular sludges.

Although acetate presented a low rate of RR2 reduction (0.31 day^{-1}), the final colour removal capacity (87.6%) was better than that achieved with formate, as shown in Fig. 4. Van der Zee et al. (2001b) reported higher RR2 reduction rates (260 mg/L) with hydrogen as the electron donor compared to acetate. The biochemical explanation for low azo dye reduction with methanogenic substrates is still largely unclear, but likely the acetoclastic methanogens are not important for reductive decolourisation compared to the hydrogenotrophic methanogens (Dos Santos et al., 2006).

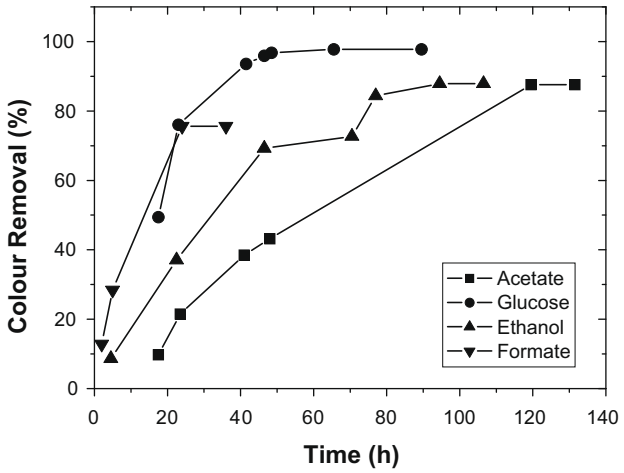

Fig. 4. Effect of different co-substrates on RR2 reduction by the anaerobic consortium 1.

Data shown in Table 2 also indicate that anaerobic inoculum 2 provided better decolourisation rates compared to inoculum 1, probably because the co-substrates ethanol and formate could be oxidized much faster by the anaerobic microorganisms and produced more electrons to reduce the dye. Therefore, the sludge source plays a role in the decolourisation rates reported.

The chemical control showed that the dye was stable to photodegradation, and endogenous and autoclaved controls showed that the colour removal was a biotic mechanism (data not shown).

3.2. AQDS as a catalyst to enhance the reductive decolourisation of azo dyes

The redox mediator AQDS (50 μM) can act as catalyst in the reductive decolourisation of azo dyes, increasing the first-order kinetic constant ($k1$) with all co-substrates tested, as shown in Table 2.

The azo dye reduction in the presence of redox mediators occurs in two steps. First, the redox mediator is biologically reduced by non-specific enzymes. Second, the electrons are chemically transferred to the azo dye, as the terminal electron acceptor, with consequent mediator regeneration (Dos Santos et al., 2007).

For co-substrate ethanol, incubated with consortium 1, AQDS addition increased the $k1$-value 3.5-fold. Interestingly for acetate, considered a poor co-substrate for dye reduction, the AQDS impact was 2.2-fold, which was comparable to the results achieved with glucose.

In most of the cases, the redox mediator also increased the final colour removal efficiency with all co-substrates tested (Table 2), indicating that, with regard to competition for electrons (including methanogenesis), AQDS channelled more electrons towards the dye reduction compared to the assays lacking this compound.

Table 2
Effect of different co-substrates and the redox mediator AQDS on RR2 reduction.

Anaerobic consortia	Electron donor (1.5 gCOD/L)	Without AQDS		With AQDS (50 μM)	
		Colour removal efficiency (%)	$k1$ (day^{-1})	Colour removal efficiency (%)	$k1$ (day^{-1})
Sludge 1	Acetate	87.6	0.31	88.6	0.70
	Glucose	97.8	1.30	96.3	2.96
	Ethanol	87.9	0.54	96.4	1.88
	Formate	75.6	1.48	92.7	4.68
Sludge 2	Ethanol	97.1	1.83	96.9	4.74
	Formate	91.9	2.74	93.3	7.59

Table 3
RR2 and CR reduction with ethanol as the electron donor (1.5 gCOD/L).

Anaerobic consortia	Azo dyes	AQDS (μM)	Colour removal efficiency (%)	K1 (day^{-1})	Standard deviation
Sludge 1	Congo Red	0	97.5	3.80	0.05
	Congo Red	50	99.3	5.12	0.07
	RR2	0	87.9	0.54	0.07
	RR2	50	96.4	1.88	0.06

The catalytic effect of only 7 μM AQDS to accelerate RR2 reduction (260 mg/L) was also found by Van der Zee et al. (2001b), increasing the decolourisation rates 2-fold with hydrogen as a co-substrate. Dos Santos et al. (2003) obtained a 5-fold increase in RR2 reduction when testing 0.012 mM AQDS with a mixture of glucose and volatile fatty acids (VFA) as a co-substrate.

Data on the catalytic effect of AQDS to accelerate CR reduction is shown in Table 3. CR reduction with ethanol as a co-substrate was faster than that found for RR2, indicating that the latter is more recalcitrant under anaerobic conditions to reductive processes.

Differences in chemical structure such as chromophores and auxochromes, the number of azo bonds, aromatic ring and substituent positions, redox potentials, etc., directly affect the biological and chemical steps involved on dye reduction (Hao et al., 2000; Rau et al., 2002; Dos Santos et al., 2007). Because of so many variables, different behaviors with respect to reductive decolourisation rates can be found.

With dye CR and sludge 1, the *k*1-values were 3.8 day^{-1} and 5.1 day^{-1}, for the bottles free and supplemented with AQDS, respectively (Table 3). Therefore, AQDS increased the dye reduction by 1.4-fold. In the presence of RR2, the *k*1-value achieved was 0.54 day^{-1}, and AQDS increased the rates by 3.5-fold. These results indicated that the catalytic effect of AQDS on dye reduction was more evident with RR2. Probably the effect was due to the higher recalcitrance of RR2 to reductive processes, compared to CR, which masked the AQDS impact.

The electron donor concentration used in batch experiments (1.5 g COD/L) was likely not a limiting factor for the reductive decolourisation of CR and RR2. The consumption of 1 mol of O_2 represents a reaction with 4 mols of electrons. If 1 mol of O_2 represents 32 g COD, 1 mol of electrons corresponds to 8 g COD. Because CR is a diazo, in theory 8 electron equivalents are required to reduce one molecule of CR dye. Therefore, to reduce 0.3 mM of the dye 2.4 mM (19.2 mg COD/L) of electrons are required. For RR2 (monoazo), 4 electron equivalents are required to reduce one molecule of RR2. Therefore, 1.2 mM (9.6 mg COD/L) of electrons is required to reduce 0.3 mM of RR2.

Although CR is a diazo, it was less recalcitrant to reductive processes, which was probably because of its linear structure compared to RR2, thereby decreasing steric hindrance effects and facilitating azo cleavage. Additionally, RR2 contained a triazine group, which generally gives a high recalcitrance to reductive processes because of the competition for electrons between nitrogen atoms from the triazine group and the nitrogen from the azo linkage (Van der Zee et al., 2001a).

In addition to the structure/activity relationship effect, azo dye reducing microorganisms and other microbes present in anaerobic consortia compete for the same electrons, which mean that it is necessary to understand the minimal electron donor (co-substrate) dosage to sustain dye reduction in batch and continuous-flow experiments (Dos Santos et al., 2006).

3.3. Effect of an AQDS gradient on the reductive decolourisation of dye RR2

The effect of an AQDS gradient on azo dye reduction was tested. Data on Table 4 suggest that increasing the catalyst concentrations had a positive effect on dye reduction. However, the impact was not that significant, indicating that low AQDS concentrations were enough to catalyse reductive decolourisation. This finding is important because AQDS addition has a cost and its concentration should be kept to a minimum.

Table 4
Reductive recolourisation of RR2 in the presence of different electron donors and AQDS concentrations.

Anaerobic consortia	Electron donor (1.5 gCOD/L)	AQDS (μM)	Colour removal efficiency (%)	*k*1 (day^{-1})	Standard deviation
Sludge 1	Acetate	0	87.6	0.31	0.02
		50	88.6	0.70	0.03
		100	88.5	0.75	0.04
Sludge 1	Glucose	0	97.8	1.30	0.06
		50	96.3	2.96	0.10
		100	96.0	3.13	0.07
Sludge 1	Formate	0	75.6	1.48	0.01
		50	92.7	4.68	0.42
		100	93.3	5.00	0.19
		200	93.1	6.09	0.12
Sludge 2	Formate	0	91.9	2.74	0,05
		50	93.3	7.59	0.23
		100	94.9	8.93	0.76
		200	93.6	10.11	0.33

When the AQDS concentration was doubled the initial concentration (Table 4) the *k*1 value increased only 1.5-fold for all the experiments. A very high *k*1 value (10.1 day^{-1}) was achieved in the bottles that contained consortium 2, formate as co-substrate and 200 μM-AQDS (Fig. 5).

Dos Santos et al. (2004) found a 1.9-fold increase in RR2 reduction compared to the AQDS-free assays while testing 24 μM of AQDS with a mixture of glucose and VFA as a cosubstrate. In the latter experiment, the concentration of 10 mM AQDS increased the *k*1-value 5.9-fold, but severely inhibited acetate conversion to methane.

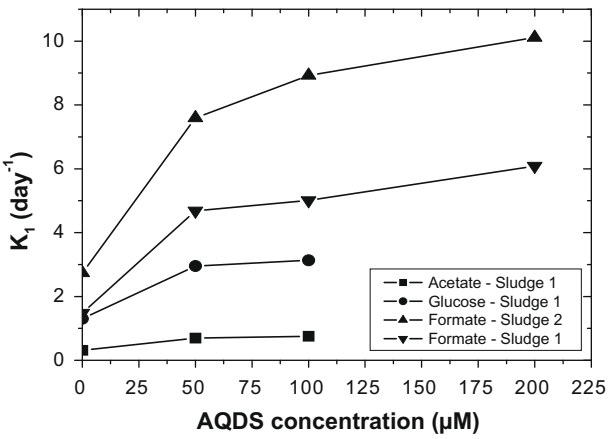

Fig. 5. Effect of an AQDS gradient on the reductive decolourisation of RR2.

Table 5
Performance of the anaerobic bioreactors in terms of colour and COD removal.

Period	1	2	3	4	5	6	7	8
End of the period (days)	12	23	85	145	169	183	198	229
OLR of co-substrate ethanol kgCOD/m^3 · d	2.5	2.5	2.5	1.0	0.5	–	0.1	1.0
Co-substrate ethanol (mgCOD/L)	833	833	833	333	166	–	33.3	333
CR (mM)	–	0.6	1.2	1.2	1.2	1.2	1.2	1.2
pH (R1)	–	8.1 ± 0.1	7.9 ± 0.2	8.1 ± 0.1	8.1 ± 0.1	8.5 ± 0	8.4 ± 0.2	8.0 ± 0.3
pH (R2)	–	8.2 ± 0	8.0 ± 0.3	8.2 ± 0.1	8.2 ± 0.1	8.6 ± 0	8.4 ± 0.2	8.1 ± 0.1
% CODsol removal (R1)	–	77.4 ± 15	49.3 ± 11	54.5 ± 33	75.9 ± 16	58.8 ± 13	56.2 ± 34	32.8 ± 11
% CODsol removal (R2)	–	83.3 ± 9	40 ± 14	60 ± 28	50.5 ± 19	44.5 ± 11	29.6 ± 6	35.5 ± 16
% Colour removal (R1)	–	94.9 ± 3	96.5 ± 7	94.2 ± 15	85.2 ± 9	23.7 ± 10	21.4 ± 5	89.9 ± 16
% Colour removal (R2)	–	99.2 ± 2	98.1 ± 4	98.4 ± 9	87.3 ± 10	17.2 ± 8	15.0 ± 12	98.5 ± 1

Room temperature was about 28 ± 2 °C during the entire experiment.

3.4. Performance of the anaerobic bioreactors in terms of colour and COD removal

Data for continuous-flow experiments are shown in Table 5. Period 1 was the acclimation time; no dye was added to the reactors. In Period 2, the azo dye CR was added at a concentration of 418 mg/L (0.6 mM), and in Period 3 twice that concentration of CR was added. The organic loading rate was changed starting in Period 4 onwards, but the same CR concentration of 1.2 mM (Table 5) was used.

High colour removal efficiencies (>90%) were achieved in the bioreactors, even for the high dye concentrations tested (1.2 mM or 836 mg/L). Isik and Sponza (2005) found 100% CR decolourisation, but the concentration was only 100 mg/L, i.e. at least 8 times lower than the concentration tested in the current experiment.

While testing the organism *D. alaskensis*, Diniz et al. (2002) found that the azo dye CR was toxic to the cells, in concentrations higher than 0.5 mM, i.e. in a concentration 2.4-fold lower than the concentration used in the present investigation (1.2 mM). No inhibition of CR or its reduced products was found in terms of cosubstrate oxidation, as VFA effluent concentration for reactors R1 and R2 was 271.7 and 294.8 mg CH3COOH/L, respectively. The results showed that it is advantageous to use anaerobic consortia compared to pure cultures, because the high microbe diversity in anaerobic consortia helps to decrease toxicity effects and enhance process stability.

AQDS addition in reactor 2 increased colour removal efficiency compared to the AQDS-free reactor 1 (Table 5). However, the difference was not as high as the rates achieved in batch experiments with CR (Table 3).

Dos Santos et al. (2005) in an experiment with RR2 (0.3 mM) found that the reactor supplemented with 25 μM of AQDS achieved colour removal efficiency up to 32% higher than the AQDS-free reactor. Such a difference was probably due to the dyes tested, since in this case it was easier to reduce the CR compared to RR2, thus masking the AQDS effect.

Brauna et al. (2009) found decolourisation efficiencies of about 80% while testing dye RR2 (20 mg/L), with the same electron donor and OLR used in the present study.

In Period 2, COD removal was 77.4% and 83.3%, for bioreactors R1 and R2, respectively. When the dye concentration was increased, which contributed to increase the influent COD, the efficiency decreased slightly to 49.3% (R1) and 40% (R2).

Singh et al. (2007) reported 46% COD removal efficiency in an experiment with azo dye Acid Orange (200 mg/L). The COD efficiency dropped to 35% when the dye concentration was increased to 300 mg/L. Brás et al. (2005) operated a lab scale UASB reactor and achieved colour removal efficiencies of 92% and 85% for azo dye Acid Orange 7 in concentrations of 60 mg/L and 300 mg/L, respectively. However, they found that COD removal efficiency decreased from 92% (60 mg/L) to 67% (300 mg/L), thereby indicating a more evident effect on COD removal capacity compared to the colour removal capacity.

In Period 4, the OLR was decreased to 1.0 kg COD/m^3 · d and the decolourisation efficiency was higher than 90% in both reactors. However, for an OLR of 0.5 kg COD/m^3 · d (Period 5), colour removal efficiencies decreased to 85.2% (R1) and 87.3% (R2).

When co-substrate ethanol was completely suspended (Period 6), the reactors efficiencies started to collapse, and the decolourisation decreased to 23.7% (R1 AQDS-free) and 17.2% (R2 AQDS-supplemented). In this period, COD efficiencies decreased to 58.8% (R1) and 44.5% (R2).

Isik and Sponza (2005), tested a lower CR concentration (100 mg/L), and achieved 99% decolourisation even when glucose was removed from the system. They also reported a decrease from 78% to 68% on COD efficiency when glucose concentration was decreased from 500 mg/L to 100 mg/L. The results of the present investigation indicate the importance of an electron donor, together with its type and concentration, to sustain dye reduction, as well as the ratio of electron donor and dye concentrations.

Brauna et al. (2009) found in continuous-flow experiments that the RR2 reduction decreased 2.5-fold when the ethanol concentration decreased from 1000 mg COD/L to 100 mg COD/L. Brás et al. (2001) achieved higher dye reduction rates operating a methanogenic sequenced batch reactor with the azo dye Acid Orange 7 (100 mg/L) when the co-substrates glucose and sucrose were increased from 300 to 3000 mg COD/L. ÓNeill et al. (2000) treated an effluent containing 0.75 g/L of azo dye Red H-E7B using two different starch concentrations (1.9 and 3.8 g/L), and showed that the decolourisation capacity of the system increased for the higher starch concentration.

In Period 7, co-substrate feeding was re-established but at low concentration (33 mg COD/L), corresponding to an OLR of 0.1 kg COD/m^3 · d. Colour removal efficiencies were 21.4% (R1) and 15.0% (R2) (Table 5). As previously mentioned, 1.2 mM of CR demands 9.6 mM electron equivalents or 76.8 mg COD/L. Therefore, the co-substrate concentration was not enough to provide electron equivalents to remove the colour in both reactors.

It is important note that the catalytic effect of AQDS on reductive decolourisation was only observed when the co-substrate (electron donor) was present. During periods of depletion or low co-substrate concentration (periods 6 and 7, respectively), the AQDS-free bioreactor R1 had a better performance than the AQDS-supplemented bioreactor R2. This indicates that the better R2 performance in the other periods was indeed due to the work of AQDS as a catalyst.

When OLR increased to 1.0 kg COD/m^3 · d, in Period 8, high colour removal efficiencies were re-established, reaching 89.9% and 98.5% for reactors R1 and R2, respectively. COD removal was lower than the values found in Period 4, likely because the microorgan-

isms were still recovering the activity lost during the co-substrate depletion period.

In general, the low COD efficiencies found were attributed to the consortium incapacity to convert the aromatic amines formed. These compounds probably did not cause toxicity to the anaerobic microorganisms, since no remarkable acetate accumulation was found. Thus, post-treatment is required to eliminate non-reduced azo dyes and the aromatic amines formed.

4. Conclusions

Colour removal efficiencies higher than 90% were found in continuous-flow experiments, even for the high CR concentrations tested. The OLR change had an evident effect on the reductive decolourisation. Therefore, it is important to establish the lowest co-substrate concentration necessary to maintain high decolourisation efficiencies.

The catalytic effect of AQDS for increasing decolourisation rates was observed with all co-substrates tested in batch experiments and low concentrations of redox mediators were enough to enhance the $k1$-values. However, AQDS addition in the continuous-flow experiments did not have the same significant effect on CR reduction, but the AQDS-supplemented bioreactor R2 presented higher stability and colour removal efficiency.

Acknowledgements

The authors would like to thank the Laboratory of Sanitation (Labosan) of the Federal University of Ceará (UFC), Christus School, and CNPq (Conselho Nacional de Desenvolvimento Científico e Tenológico) for the PhD scholarship and financial support (Process 470310/2007-3 from Edital Universal). Finally, we thank the assistance of the Laboratory of Water (Lanágua) of UFC.

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