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Impact of the redox mediator sodium anthraquinone-2,6-disulphonate (AQDS) on the reductive decolourisation of the azo dye Reactive Red 2 (RR2) in one- and two-stage anaerobic systems

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HIGHLIGHTS

- Sulphonated dye reduction in one- and two-stage anaerobic systems.
- Impact of phase separation on electron transfer capacity.
- Impact of redox mediators in one- and two-stage anaerobic systems.
- Impact of acidogenic phase in the overall colour removal.
- Possibility of application to other reductive processes.

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ABSTRACT

This work assessed the impact of the redox mediator sodium anthraquinone-2,6-disulphonate (AQDS) on the reductive decolourisation of the azo dye Reactive Red 2 (RR2) in one- and two-stage anaerobic systems (R_1 and R_2, respectively). The two-stage system achieved better colour removal efficiencies (52–62%) than the single-stage system (23–33%) in the absence of AQDS. Addition of AQDS accelerated the electrons transfer from the substrate (ethanol) to the dye, which increased the colour removal efficiency of both anaerobic systems (~85%). Finally, the impact of acidogenic and methanogenic phases separation was masked by AQDS supplementation.

1. Introduction

Although recent statistics on global production of dyes are not readily available (Cervantes and dos Santos, 2011), it is estimated that over 10,000 tons of dyes are produced annually in the world, amongst which the azo dyes are the most employed in industrial scale (>50%), followed by the anthraquinone and phthalocyanine dyes (Forgacs et al., 2004). Depending on the dye class, the percentage of dye that remains unfixed to the fibre during the dyeing process varies from 5% up to 50% on a weight base (Cervantes and dos Santos, 2011). Therefore, the release of dye-containing effluents into surface waters represents a serious environmental problem and a public health concern (dos Santos et al., 2007) since these compounds and their breakdown products are toxic, mutagenic or carcinogenic (Weisburger, 2002).

Amongst the different decolourisation methods, biological treatment has called attention for being economically attractive. However, since oxygen is a more effective electron acceptor than azo dyes, colour removal by aerobic bacteria, such as those commonly present in activated sludge systems, is normally low (10–30%) (dos Santos et al., 2007), which is mainly associated with the dye adsorption onto the sludge (Alinsafi et al., 2006). On the other hand, under anaerobic conditions, effective dye decolourisation can be reached (van der Zee et al., 2001b).

This process is also referred as dye reduction, being the azo dye reduction biochemistry mostly reported in literature. The azo bond (–N=N–) cleavage involves four electrons (reducing equivalents) transfer to the azo dye, which acts as a final electron acceptor.

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After this cleavage, aromatic amines are produced (dos Santos et al., 2007).

Since dye reduction competes with methanogenesis for the same electrons generated upon electron donor oxidation (Cervantes et al., 2005, 2008; dos Santos et al., 2006), it seems that a two-stage anaerobic system, in which acidogenic and methanogenic phases are separated, could be an interesting option to enhance colour removal, i.e., the probability of the electrons to be channelled to dye reduction would be higher.

In addition, decolourisation of recalcitrant dyes in anaerobic reactors is normally a slow process, which requires long hydraulic retention times (HRT) to reach a satisfactory colour removal extent. Thus, the application of redox mediator compounds such as flavin-based vitamins and quinones present in humus in anaerobic treatment of dye-containing effluents can enhance decolourisation rates (Baêta et al., 2012; van der Zee and Cervantes, 2009).

Although two-stage anaerobic degradation has been successfully applied to treatment of several complex industrial wastewaters (Demirel and Yenigün, 2002; Ke et al., 2005), reports on this technology for dye-containing wastewaters decolourisation are still relatively scarce in literature (Bhattacharyya and Singh, 2010, 2008; Chinwetkitvanich et al., 2000; Firmino et al., 2010; Gnanapragasam et al., 2010; Rai et al., 2007; Senthilkumar et al., 2011a,b; Talarposhti et al., 2001). Additionally, none of them assessed the impact of redox mediator compounds supplementation on decolourisation performance of two-stage anaerobic systems.

Hence, the main objective of this paper was to assess and compare the impact of the redox mediator sodium anthraquinone-2,6-disulphonate (AQDS) supplementation on the reductive decolourisation of the sulphonated dichlorotriazine azo dye Reactive Red 2 (RR2) in one- and two-stage anaerobic systems.

2. Methods

2.1. Reactors

The up-flow anaerobic sludge blanket (UASB) reactors were made of PVC tubes and connections for sewage. The one-stage system (R_1) (Fig. 1a) consisted of a single reactor (V = 5.2 L), and the two-stage system (R_2) (Fig. 1b) consisted of an acidogenic reactor (R_A) (V = 1.1 L), a settler and a methanogenic reactor (R_M) (V = 5.1 L). The reactors were inoculated with an anaerobic sludge from a brewery mesophilic UASB reactor (Industrial District, Ceará, Brazil) at a final concentration of approximately 30 g VSS L^{-1}.

With the exception of R_A, the other reactors had a modified gas–solid-liquid separator (Y-shaped) (Cavalcanti, 2003). In order to avoid the formation of preferential flow paths or short circuiting flows trough the sludge blanket and facilitate the biogas release, avoiding the piston effect (sludge blanket rise due to entrapped biogas), a slow stirrer (5 rpm) was installed in the reactors (Leitão, 2004).

The influent was stored at 4 °C, and the reactors were operated at room temperature of approximately 27 °C. The biogas produced was collected and washed in a NaOH solution (0.5 N), and, then, methane was measured by a Mariotte flask (liquid displacement method).

2.2. Synthetic textile wastewater

The dye used was Reactive Red 2 (RR2) (50% purity, Sigma–Aldrich, USA), a sulphonated dichlorotriazine monoazo dye. The electron donor was ethanol (99.8% purity, Dinâmica, Brazil), and the basal medium composition was according to Firmino et al. (2010). To keep the pH around 7.0, the wastewater was buffered with sodium bicarbonate ($NaHCO_3$) in the proportion of 1 g $NaHCO_3$ to each 1 g COD ethanol. In some experimental periods, the anaerobic systems were supplemented with sodium anthraquinone-2,6-disulphonate (AQDS) (Aldrich, USA), a redox mediator model compound, in order to assess its impact on colour removal efficiencies. All chemicals were used as purchased without further purification.

2.3. Experimental procedure

The experiment, in which the one- and two-stage anaerobic systems treated a synthetic wastewater containing the reactive azo dye RR2, was divided in four periods, as detailed in Table 1.

Initially, the anaerobic systems were fed with 100 mg L^{-1} of RR2 (period I), and, subsequently, the dye concentration was increased to 200 mg L^{-1} (period II). In period III, in order to evaluate the influence of the redox mediator AQDS on the performance of anaerobic reactors, 25 μM of this compound was added to the composition of the synthetic wastewater. Finally, in period IV, the concentration of AQDS was increased to 50 μM in order to

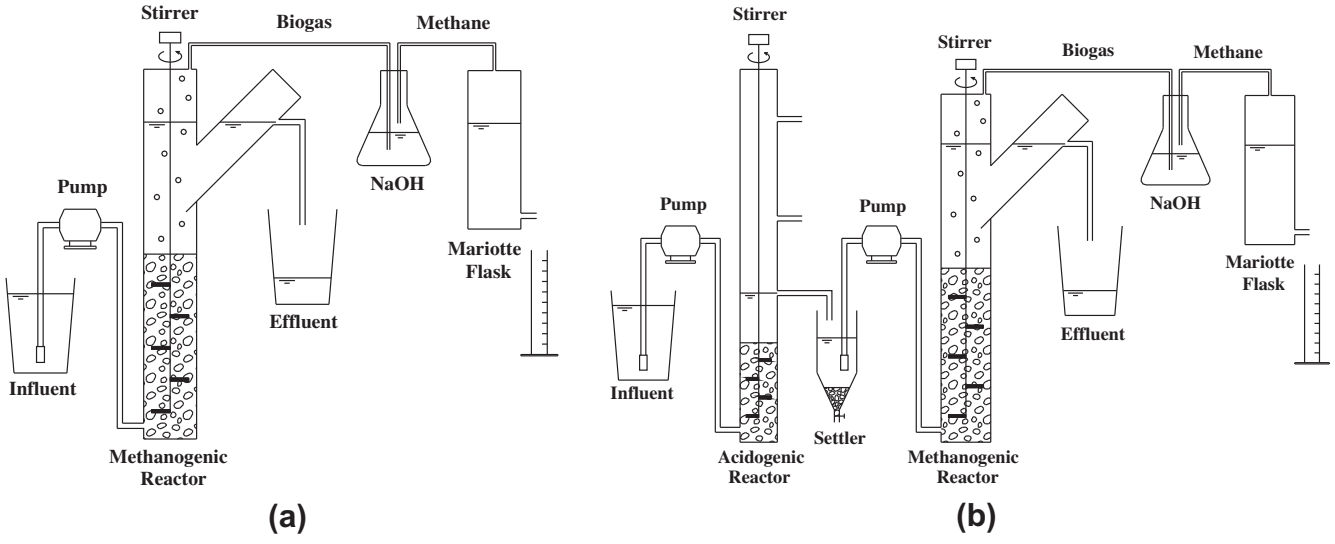

Fig. 1. Schematic of the one-stage (a) and two-stage (b) anaerobic systems.

Table 1
Operational parameters of the reactors over the experimental periods.

Operational parameters				
Period	I	II	III	IV
End of period (days)	22	59	73	89
HRT (h) R_1	12	12	12	12
HRT (h) R_A	3	3	3	3
HRT (h) R_M	9	9	9	9
Substrate (g COD·L^{-1})	1.0	1.0	1.0	1.0
RR2 (mg·L^{-1})	100	200	200	200
AQDS (μM)	-	-	25	50

HRT, hydraulic retention time; R_1, one-stage system; R_A, acidogenic reactor; R_M, methanogenic reactor; COD, chemical oxygen demand; RR2, Reactive Red 2; AQDS, sodium anthraquinone-2,6-disulphonate.

assess the effect of the concentration of the redox mediator on RR2 anaerobic decolourisation.

2.4. Analyses

Colour was usually analysed three times a week and determined photometrically (Thermo – Nicolet Evolution 100) by using a single wavelength method (dos Santos et al., 2007) based on dos Santos et al. (2003, 2005, 2006). The absorbance was read at wavelength (λ) of 513 nm, i.e., at wavelength whose absorbance is maximum. Samples were previously diluted (1:5) in a phosphate buffer (10.86 g/L $NaH_2PO_4{\cdot}2H_2O$ and 5.98 g/L $Na_2HPO_4{\cdot}2H_2O$) and, then, centrifuged for 2 min at 13,000 rpm (Eppendorf – Mini Spin).

COD, pH, alkalinity and volatile fatty acids (VFA) were usually analysed twice a week. COD was determined photometrically (Thermo – Nicolet Evolution 100) by the closed reflux method, while pH was determined by potentiometric method (Digimed – DM 20) and total alkalinity by titrimetric method, all of them according to Standard Methods (APHA, 2005). VFA were determined using Kapp titrimetric method (Buchauer, 1998).

2.5. Statistical methods

SigmaStat 3.5 computer program was used for the statistical analysis of the data, being applied the Mann–Whitney Rank Sum and Kruskal–Wallis ANOVA on Ranks tests, non-parametric procedures which do not require a specific data distribution, to compare the performance of both systems during the different experimental periods. The results of the tests were evaluated according to the p-value. If $p \leqslant 0.050$, the null hypothesis is rejected, i.e., the data groups are considered statistically different.

3. Results and discussion

3.1. Colour removal in one- and two-stage anaerobic systems in the absence of AQDS

At the beginning of period I, higher decolourisation efficiencies were observed (Fig. 2), probably due to the initial RR2 adsorption onto the sludge blanket (dos Santos et al., 2003). Subsequently, these values decreased gradually in the period (Fig. 2), during which the two-stage system (R_2) was clearly more efficient than the one-stage system (R_1) ($p < 0.001$), reaching an almost 2-fold higher average of decolourisation (~62%) (Table 2). Additionally, the acidogenic reactor (R_A) was responsible for 38% of total efficiency of R_2 (Fig. 3).

By increasing the dye concentration from 100 to 200 mg L^{-1} (period II), the average efficiency of both systems decreased by approximately 10% (Table 2). However, in terms of dye rate removal, R_1 and R_2 could reduce RR2 at a higher theoretical rate

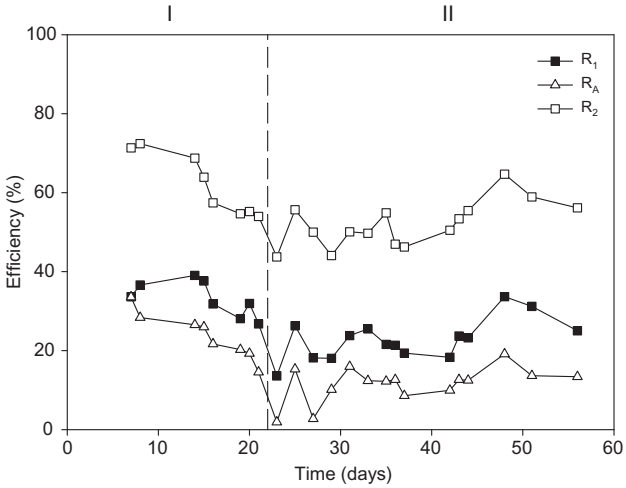

Fig. 2. Colour removal efficiency of the azo dye RR2 in the one- (R_1) and two-stage ($R_2 = R_A + R_M$) anaerobic systems during periods I and II.

(for a total HRT of 12 h), i.e., 3.81 and 8.67 mg·L^{-1}·h^{-1}, respectively, in period II in comparison with 2.77 and 5.52 mg L^{-1} h^{-1} in period I. The initial dye concentration increase might have enhanced decolourisation kinetics as observed by Beydilli and Pavlostathis (2005) when they increased the initial RR2 concentration from 50 to 300 mg L^{-1} in batch experiments. Nonetheless, R_2 remained (~2.5-fold) more efficient than R_1 ($p < 0.001$), and R_A contributed only 22% to total decolourisation of the two-stage system (Fig. 3).

These results contradict the findings of Firmino et al. (2010), who observed no remarkable differences between the one- and two-stage systems used in the decolourisation of the azo dye Congo Red, even when high concentrations were tested (840 mg L^{-1}). According to those authors, the fact that the dye Congo Red presents a linear molecular structure allowed it to be easily reduced. Hence, the application of two-stage systems might be more suitable and, therefore, more efficient for effluents which contain more recalcitrant dyes such as RR2. Since this dye contains a triazine group, the reductive processes becomes more difficult due to the competition for the electrons between nitrogen atoms from the triazine group and the nitrogen from the azo linkage (Costa et al., 2010; van der Zee et al., 2001b).

dos Santos et al. (2005), using an expanded granular sludge bed (EGSB) reactor (HRT = 10 h) to treat a synthetic wastewater containing 520 mg L^{-1} of hydrolysed RR2, achieved average colour removal efficiencies of approximately 56% under mesophilic conditions (30 °C). In contrast, van der Zee et al. (2001a) operated a UASB reactor (HRT = 6 h) fed with a synthetic wastewater containing only 200 mg L^{-1} of non-hydrolysed RR2 and obtained decolourisation efficiencies of 20–30%. According to the latter authors, the reactor showed a high operational instability and collapsed after 32 days of dye introduction into the reactor influent, mainly due to RR2 toxicity – caused by the non-hydrolysed chlorotriazine group, which severely inhibited biological activity of the reactor sludge. However, non-hydrolysed RR2 has also been used in the current experiment and no toxicity inhibition was evidenced in either system used (Section 3.3).

Finally, analysing the acidogenic reactor performance, lower decolourisation efficiencies were reached when compared to previous studies, in which the acidogenic stage was usually the main stage responsible for the total decolourisation obtained by the two-stage system (Bhattacharyya and Singh, 2010, 2008; Chinwetkitvanich et al., 2000; Firmino et al., 2010; Rai et al., 2007; Talarposhti et al., 2001). This might be related to the steric hindrance of RR2 molecule, which makes electrons transfer from

Table 2
Operational performance of the reactors over the experimental periods.

Operational performance				
Period	I	II	III	IV
Colour removal (%) R_1	33.2 (4.4)	22.9 (5.2)	85.2 (1.3)	85.7 (1.2)
Colour removal (%) R_A	23.8 (6.0)	11.6 (4.5)	33.9 (3.2)	36.3 (8.0)
Colour removal (%) R_2	62.2 (7.8)	52.0 (5.8)	84.1 (1.6)	86.0 (2.3)
Influent COD ($mg \cdot L^{-1}$) R_1	1046 (43)	1290 (175)	1222 (48)	1240 (32)
Effluent COD ($mg\,L^{-1}$) R_1	278 (117)	414 (85)	448 (19)	439 (23)
COD removal (%) R_1	72.5 (10.3)	67.1 (4.7)	63.4 (2.6)	64.3 (1.9)
Influent COD ($mg \cdot L^{-1}$) R_2	1046 (43)	1290 (175)	1222 (48)	1240 (32)
Effluent COD ($mg\,L^{-1}$) R_2	447 (108)	429 (60)	427 (20)	427 (32)
COD removal (%) R_A	10.1 (3.4)	26.0 (7.6)	30.3 (5.8)	34.9 (1.7)
COD removal (%) R_2	56.5 (9.2)	66.7 (3.9)	65.3 (2.2)	65.1 (2.8)
pH R_1	7.8 (0.1)	7.9 (0.1)	7.8 (0.2)	7.8 (0.0)
pH R_A	7.4 (0.2)	7.4 (0.1)	7.3 (0.1)	7.2 (0.1)
pH R_M	7.9 (0.0)	8.0 (0.1)	7.8 (0.0)	7.8 (0.1)
BA ($mg\,L^{-1}$) R_1	552.2 (40.7)	563.1 (53.3)	529.7 (25.1)	531.8 (28.7)
BA ($mg\,L^{-1}$) R_A	298.2 (61.8)	316.3 (26.2)	292.3 (26.1)	284.5 (30.6)
BA ($mg\,L^{-1}$) R_M	494.3 (40.0)	576.5 (31.0)	522.9 (37.0)	499.4 (26.5)
VFA ($mg\,L^{-1}$) R_1	115.7 (50.4)	85.7 (58.2)	75.6 (12.1)	72.3 (9.0)
VFA ($mg\,L^{-1}$) R_A	468.5 (82.8)	383.3 (135.0)	290.5 (84.2)	319.0 (54.6)
VFA ($mg\,L^{-1}$) R_M	176.5 (62.8)	67.1 (23.2)	79.2 (11.1)	97.2 (21.4)
VFA/TA R_1	0.19 (0.09)	0.14 (0.10)	0.13 (0.02)	0.13 (0.02)
VFA/TA R_A	0.89 (0.19)	0.74 (0.22)	0.66 (0.12)	0.72 (0.06)
VFA/TA R_M	0.30 (0.11)	0.11 (0.04)	0.14 (0.01)	0.18 (0.03)

The standard deviation is shown in parenthesis.
R_1, one-stage system; R_2, two-stage system; R_A, acidogenic reactor; R_M, methanogenic reactor; COD, chemical oxygen demand; BA, bicarbonate alkalinity; VFA, volatile fatty acids; TA, total alkalinity.

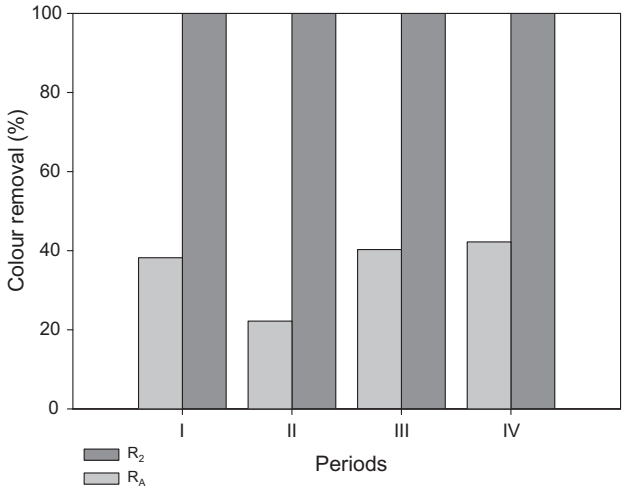

Fig. 3. Relative colour removal performance of the acidogenic reactor (R_A) compared to the overall colour removal in the two-stage anaerobic system (R_2).

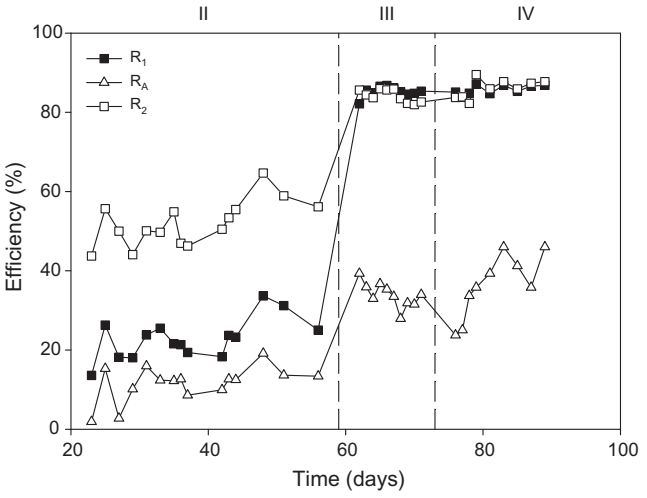

Fig. 4. Colour removal efficiency of the azo dye RR2 in the one- (R_1) and two-stage ($R_2 = R_A + R_M$) anaerobic systems during periods II to IV.

the substrate to the dye more difficult. Therefore, the short HRT (3 h) was not sufficient to allow a higher decolourisation. In agreement with that, dos Santos et al. (2005) found that the decrease in the HRT of their EGSB reactor from 10 to 5 and, then, to 2.5 h, reduced the RR2 colour removal efficiencies from 56% to 37% and to 13%, respectively, therefore suggesting that the short HRTs used were not long enough to allow dye reduction satisfactorily.

According to Bhattacharyya and Singh (2010), the application of two-stage anaerobic systems to treat dye-containing wastewaters is a way of overcoming the inhibitory effect of dyes since the acidogenic reactor works as a detoxifier, preventing the more sensitive methanogens from being in direct contact with the toxic waste. However, the most likely hypothesis of utilizing a two-stage reactor is based on the fact that methanogenesis competes with the dye for the same electrons generated upon substrate oxidation (Cervantes et al., 2005, 2008; dos Santos et al., 2006). Consequently, by separating the two processes, the probability of the electrons to be channelled to the dye would be higher, which would enhance colour removal. Hence, as RR2 is a more recalcitrant dye, the present work succeeded in demonstrating the impact acidogenic and methanogenic phases separation on anaerobic decolourisation.

3.2. Colour removal in one- and two-stage anaerobic systems in the presence of AQDS

In period III, 25 μM of the redox mediator AQDS were added into the influent, and the average colour removal efficiencies of both systems increased considerably to approximately 85% (Table 2). As a result, no statistical difference was observed in their decolourisation performance ($p = 0.212$).

However, the impact of the redox mediator compound on colour removal was much more remarkable in R_1 than in R_2 (Fig. 4), i.e., the average decolourisation efficiency of the single-stage system in period III was 3.7-fold higher than in period II whereas the efficiency of the two-stage system was only 1.7-fold higher than the AQDS-free period. Additionally, R_A achieved a 2.9-fold higher average decolourisation efficiency (~34%) when compared to period II (Table 2) and was responsible for approximately 40% of the total colour removal obtained by R_2 (Fig. 3).

These results are supported by those found by Costa et al. (2010) in a RR2 (~185 mg L^{-1}) decolourisation batch assay with ethanol as co-substrate and the same sludge source used in the current experiment. These authors reported that the addition of 50 μM of AQDS increased 3.5-fold the decolourisation first-order kinetic constant (k_1).

Accordingly, there was a marked impact of the redox mediator on the electron transfer from the electron donor (ethanol) to the azo RR2 (final electron acceptor), which therefore increased the colour removal efficiency of the anaerobic systems R_1 and R_2. This is also in accordance to the results presented by dos Santos et al. (2005), who utilized expanded granular sludge bed (EGSB) reactors (HRT = 10 h) to decolourise the azo dye RR2 and verified that the reactor also supplemented with AQDS (25 μM) achieved an 88% decolourisation, while the AQDS-free reactor only reached 56%. Nevertheless, in the present study, AQDS addition into the influent masked the effect of the acidogenic and methanogenic phases separation in the two-stage system (R_2).

However, the impact of redox mediators is not always large: sometimes the accelerating effect is small, sometimes absent, and in rare cases even adverse since this impact reflects the differences between the investigated systems, their conditions, the dye/mediator/biomass-combinations used, etc. (van der Zee and Cervantes, 2009). For instance, Braúna et al. (2009) observed low catalytic effects of AQDS (12.5 and 25 μM) on decolourisation of RR2 (from 20 to 80 mg/L). The authors attributed this result to the low dye concentration tested associated to the high biomass concentration in the reactor (30 g VSS L^{-1}), which may have conducted the decolourisation reactions to follow a zero-order kinetics and, therefore, masked the AQDS effect. Additionally, for non recalcitrant dyes such as Congo Red, which has a linear molecule structure that allows easy chromophore reduction, the addition of a redox mediator compound in an anaerobic reactor, also fed with the same sludge source used in the present investigation, was not very evident on colour removal, even when very high dye concentrations were applied (>800 mg/L) (Costa et al., 2010).

By increasing AQDS concentration from 25 to 50 μM (period IV), the colour removal performance of both systems remained the same as the previous period (Fig. 4), reaching similar average efficiencies ($p > 0.050$) (Table 2). Consequently, in period IV, there was also no statistical difference between R_1 and R_2 decolourisation performances ($p = 0.596$) (Fig. 4). Even for the acidogenic reactor (R_A), which was operated at a short HRT (3 h), the redox mediator concentration increase (from 25 to 50 μM) did not enhance reactor colour removal efficiency ($p = 0.270$) (Table 2), although R_A was responsible for 42% of the overall decolourisation of R_2 (Fig. 3). However, since a higher AQDS concentration did not significantly increase the decolourisation efficiencies, it might indicate a saturation kinetics as observed by Field and Brady (2003).

Costa et al. (2010) tested the effect of AQDS gradient on RR2 reduction (~185 mg L^{-1}), and their results suggested that the redox mediator concentration increase had a positive effect on dye reduction. However, the impact was not significant, indicating that low AQDS concentrations were enough to catalyse reductive decolourisation.

Furthermore, Cervantes et al. (2001), while studying the Acid Orange 7 decolourisation by using a UASB reactor (HRT = 6 h), observed that an almost complete decolourisation (>97%) could be achieved with the lowest AQDS concentration tested (3 μM), i.e., a molar ratio of AQDS/AO7 of about 0.01 was sufficient to achieve an efficient decolourisation process. This seems to be in accordance with the results found in the present study since a molar ratio of AQDS/RR2 of approximately 0.08 (period III) was sufficient to reach efficiencies over 85% in R_1 and R_2.

On the other hand, van der Zee et al. (2001a), also with a UASB reactor (HRT = 6 h), observed that RR2 colour removal efficiencies increased from about 25–98% with a gradual increase in AQDS concentration (from 0 to 155 μM).

3.3. COD removal and operational stability

Operational data for reactor performances are shown in Table 2, in which a good operational stability can be observed for both systems. The average pH values achieved are in accordance with Pearce et al. (2003), who affirmed the optimum pH for colour removal is, in general, neutral or slightly alkaline. Low VFA concentrations were found in reactors R_1 and R_M during the whole experiment (Table 2), which indicates that the microbial activity might not have been inhibited by toxicity of the dye or its by-products (aromatic amines) resulted from anaerobic reduction. On the other hand, there was VFA accumulation in R_A (VFA/TA > 0.65) (Table 2). However, this behaviour was expected since the acidogenic reactor is responsible for converting more complexes substrates into low-chain organic acids.

In relation to COD removal, unexpectedly, R_1 achieved a higher average efficiency than R_2 in period I ($p = 0.038$) (Table 2) with no apparent reason since no evidence of toxicity inhibition was verified as mentioned above. From period II onwards, there was no significant statistical difference between the overall average COD removal efficiencies achieved by R_1 and R_2 ($p > 0.310$), which ranged between 63.4% and 67.1% (Table 2).

In contrast to that, previous studies reported that a two-stage system performed better than a single-stage system in terms of total COD removal throughout their experiment even under different operational conditions, such as different dye and substrate concentrations and different HRTs (Bhattacharyya and Singh, 2010; Firmino et al., 2010).

Furthermore, neither the increase in dye concentration from 100 to 200 mg L^{-1} nor the AQDS supplementation (25 or 50 μM) directly affected the COD removal of the anaerobic systems, since no significant statistical difference in their performance was observed along the whole experiment for R_1 ($p = 0.072$) and, from period II to IV, for R_2 ($p = 0.427$), i.e., it seems that there was no problem with electron donor (ethanol) conversion.

Therefore, since the same degree of COD removal was verified during the different experimental periods, it might indicate that the reducing equivalents were generated at a similar rate in both systems. Apparently, the difference in the decolourisation efficiencies was not related to the difference in the production rates of reducing equivalents. Consequently, the higher degree of colour removal could be attributed to the impact of either the redox mediator compound on electron shuttling (dos Santos et al., 2005) or the separation of the acidogenic and methanogenic phases during the AQDS-free periods.dos Santos et al. (2003) also stated that the co-substrate (a glucose-VFA mixture) conversion was not affected even when high hydrolysed RR2 concentrations (up to 1.25 g L^{-1}) were imposed to their thermophilic EGSB reactors (HRT = 10 h) when supplemented or not with AQDS (6 or 24 μM). In contrast, van der Zee et al. (2001a) had their mesophilic UASB reactor (HRT = 6 h) collapse after 53 days of experiment, resulting in VFA (co-substrate) removal efficiencies as low as 5–10% because the

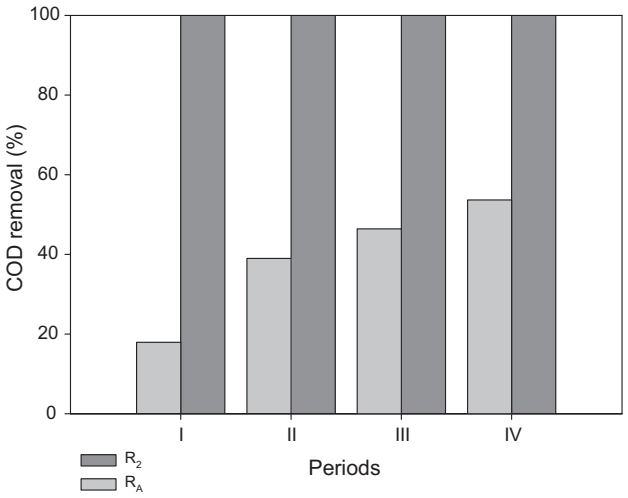

Fig. 5. Relative COD removal performance of the acidogenic reactor (R_A) compared to the overall COD removal in the two-stage anaerobic system (R_2).

reactor was fed with non-hydrolysed RR2 (200 mg L-1). However, the co-substrate oxidation was recovered (>95%) after the reactor was supplied with 19 μM of AQDS. As mentioned in Section 3.1, the present work also used non-hydrolysed RR2, but no inhibition was observed.

Concerning the acidogenic reactor (R_A) performance, in period I, low COD removal efficiencies were obtained (~10%) (Table 2), corresponding to approximately only 18% of the overall COD removal reached by R_2 (Fig. 5). Probably, the anaerobic microbiota needed a longer period of time to be adapted to the operational conditions since higher efficiencies were obtained in the subsequent experimental periods (Table 2).

From period II onwards, although R_A was operated at a HRT of only 3 h, the average COD removal efficiencies increased gradually from approximately 10% (period I) to 26%, 305% and 35% in periods II, III and IV, respectively (Table 2), i.e., from 39% to 54% of the total COD removed by the two-stage system (Fig. 5). As well, the average VFA concentrations in R_A tended to decrease along the experimental periods.

These results are in agreement to those found by Bhattacharyya and Singh (2010), who observed that 30–50% of influent COD (sucrose as co-substrate) was removed by the acidogenic reactors even when operated at short HRTs. However, according to these authors, reactors tend to be methanogenic even at a HRT as low as 3 h, particularly when an easily biodegradable co-substrate such as sucrose is used as the electron donor. Consequently, a significant fraction of the total influent COD can be removed by the first stage of the system.

Conversely, some papers report that, depending on the experimental conditions, the acidogenic stage could reach COD removals from 10% to almost 60% when more complex electron donors were used, such as sago (Gnanapragasam et al., 2010; Senthilkumar et al., 2011a, b) or tapioca (Chinwetkitvanich et al., 2000).

Finally, it is worth mentioning that the residual COD, in all experimental periods, was still very high. Additionally, dye decolourisation by-products are normally toxic, carcinogenic or, even, mutagenic. Therefore, a post-treatment for the anaerobic effluents is required.

4. Conclusions

The two-stage system achieved better colour removal efficiencies than the single- stage system in the absence of the redox mediator compound.

The addition of AQDS accelerated the electrons transfer from the substrate to the dye, which increased considerably the colour removal efficiency of both anaerobic systems.

The impact of the separation of the acidogenic and methanogenic phases was masked by the supplementation of AQDS into the systems.

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