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APPLICABILITY OF ANTHRAQUINONE-2,6-DISULFONATE (AQDS) TO ENHANCE COLOUR REMOVAL IN MESOPHILIC UASB REACTORS TREATING TEXTILE WASTEWATER

P. I. M. Firmino, M. E. R. da Silva, F. S. B. Mota and A. B. dos Santos*

Department of Hydraulic and Environmental Engineering, Federal University of Ceará,
Phone/Fax: + (55) (85) 3366-9490, Campus do Pici, Bloco 713, Pici, CEP: 60455-900, Fortaleza - Ceará, Brazil.
E-mail: andre23@ufc.br

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Abstract - This work assessed the applicability of the redox mediator anthraquinone-2,6-disulfonate (AQDS) to enhance colour removal in mesophilic UASB reactors treating textile wastewater under different operational conditions, such as different electron donor (ethanol) concentrations and different HRT. The anaerobic reactors were able to remove reasonably well the colour of the textile wastewater (35-63%) even when operated with a relatively short HRT (6 h), being a good option for textile effluents pre-treatment. Aditionally, colour removal efficiency was positively influenced not only by the addition of ethanol as external electron donor, but also by the initial wastewater absorbance. Although the applicability of AQDS is reported in the literature to enhance remarkably colour removal from synthetic dye-containing wastewaters, especially for recalcitrant azo dyes, the same effect was not evident in the present study with the textile wastewater tested, since the reactors did not show significant differences on decolourisation capacity.
Keywords: Anaerobic treatment; Colour removal; Redox mediator; Textile wastewater

INTRODUCTION

With the increased demand for textile products in the last decades, a proportional increase in wastewater generation was observed, via which a large amount of chemicals are released into surface waters, representing a serious environmental problem and a public health concern (Dos Santos *et al*., 2007).

One of the main characteristics of textile wastewaters is that they are highly coloured, mainly due to the dyes applied in the dyeing step (Banat *et al*., 1996). 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 basis (Srinivasan and Murthy, 2009). It is estimated that over 10,000 tons of dyes are produced annually in the world, amongst which the azo type are the most widely employed on an industrial scale (>50%), followed by the anthraquinone and phthalocyanine dyes (Forgacs *et al*., 2004).

Hence, the release of dye-containing effluents into the environment is undesirable, not only for affecting the aesthetics, the water transparency and the gas solubility of water bodies, but also because many of these compounds and their breakdown products are toxic, mutagenic or carcinogenic (Banat *et al*., 1996; Pandey *et al*., 2007; Weisburger, 2002).

However, colour removal of these compounds is still one of the most difficult tasks faced by wastewaters treatment plants of textile factories since dyes and pigments are designed to be resistant to light, microbial activity, ozone and other adverse

*To whom correspondence should be addressed

environmental conditions (Forgacs *et al*., 2004), allowing them to remain in the environment for an extended period of time. For instance, the half-life of the hydrolysed dye Reactive Blue 19 is about 46 years at pH 7 and 25°C (Hao *et al*., 2000).

Different decolourisation methods have been researched. Although some physical and chemical methods such as adsorption, membrane filtration, flocculation, electrolysis and advanced oxidation can be very effective, most of them present limitations such as excessive chemical demands, sludge disposal, high operational cost and strict operational conditions, which restrict their practical application (Li *et al*., 2009).

In contrast, biological methods have been widely used in practice because they are economically attractive, easy to operate and generally considered to be environmentally friendly because they can lead to complete mineralization of organic pollutants (Li *et al*., 2009; Pandey *et al*., 2007). However, colour removal by aerobic bacteria, such as those commonly present in activated sludge systems, is normally low (Dos Santos *et al*., 2007) and is mainly associated with dye adsorption in the sludge (Alinsafi *et al*., 2006). On the other hand, under anaerobic conditions, effective dye decolourisation can be reached (Costa *et al*., 2010; Dos Santos *et al*., 2005b; Firmino *et al*., 2010).

However, decolourisation of recalcitrant dyes in anaerobic reactors is normally a slow process, which requires long hydraulic retention times (HRT) to reach a satisfactory extent of colour removal (Li *et al*., 2009). Thus, the application of redox mediator compounds, such as flavin-based vitamins and quinones present in humus in the anaerobic treatment of dye-containing effluents, can enhance decolourisation rates (Cervantes *et al*., 2001; Van der Zee *et al*., 2001; Van der Zee and Cervantes, 2009).

Finally, most studies on textile wastewater decolourisation in anaerobic reactors supplemented with redox mediators have been carried out with synthetic wastewaters, mainly with azo dyes, and they usually present a remarkable impact of redox mediators on colour removal (Dos Santos *et al*., 2007; Van der Zee and Villaverde, 2005). Hence, it is important to perform experiments with real textile effluents in order to investigate whether these findings can also be applied in the presence of different dyes, electron acceptors and other pollutants. The present work assessed the applicability of anthraquinone-2,6-disulfonate (AQDS) to enhance colour removal in mesophilic upflow anaerobic sludge blanket (UASB) reactors treating textile wastewater under different operational conditions, such as different electron donor (ethanol) concentrations and different HRT.

MATERIALS AND METHODS

Reactors

Two acrylic UASB reactors (R_1 and R_2) were used in these experiments (Figure 1) and inoculated with anaerobic sludge from a brewery mesophilic UASB reactor (Industrial District, Ceará, Brazil). Their internal diameter, total height and working volume were 8.0 cm, 55 cm and 2.6 L, respectively. The sludge volume added to the reactors provided a sludge concentration of approximately 30 g of volatile suspended solids per liter (VSS/L). The influent was stored at 4°C and the reactors were operated at a room temperature of approximately 27°C.

In order to avoid the formation of preferential flow paths or short circuiting flows through the sludge blanket and facilitate the biogas release, thus avoiding the piston effect (sludge blanket rise due to entrapped biogas), a slow stirrer (5 rpm) was installed in the reactors.

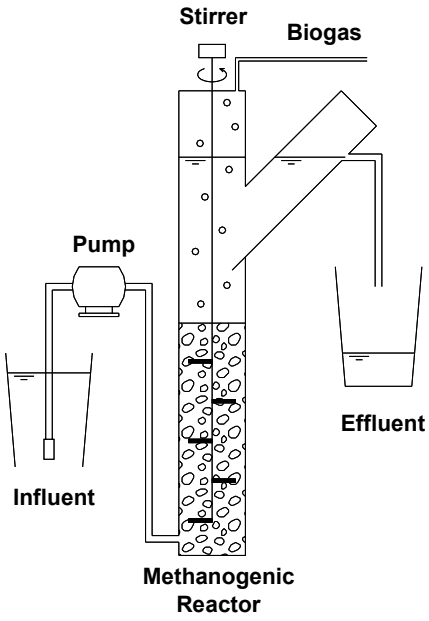

Figure 1: Experimental set-up of the UASB reactors

Textile Wastewater

The textile wastewater was collected weekly from a cotton-processing factory (Industrial District, Ceará, Brazil) whose continuous dyeing process uses

many different reactive dyes such as vinyl sulphonate, monochloro triazine and bifunctional dyes, several disperse dyes and sulphur dyes such as the sulphur black dye, which mostly belong to either the azo or anthraquinone classes. The normally alkaline pH (~10) was previously adjusted to 7.0 with sulphuric acid (H_2SO_4), then the wastewater was diluted when necessary and, finally, electrons donor, nutrients (basal medium) and buffer were added.

The electron donor was ethanol (99.8% purity, Dinâmica, Brazil) and the basal medium composition was prepared 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 chemical oxygen demand (COD) of ethanol. In some experimental periods, R_1 was supplemented with anthraquinone-2,6-disulfonate (AQDS) (Aldrich, USA), a redox mediator model compound, in order to assess its impact on colour removal efficiencies.

Experimental Procedure

The experiments were conducted in five different periods (Table 1), including reactor start-up (acclimatization period) (period I). After reaching steady operational conditions during the start-up period, the reactors were fed with the textile wastewater diluted to 50% with distilled water (period II) in order to avoid a possible microbial inhibition. After verifying reactor stability, both were then fed with undiluted wastewater and only R_1 was supplemented with AQDS (period III). In period IV, the reactors were not provided with ethanol in order to assess the real need of an external electron donor to achieve a good colour removal. Finally, in period V, the HRT was reduced from 12 to 6 h and the reactors were supplemented with 0.5 g COD/L of ethanol to keep the same organic loading rate (OLR) for the external electron donor.

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

Operational parameters					
Period	**I**	**II**	**III**	**IV**	**V**
End of period (days)	44	81	110	152	168
HRT (h) R_1	12	12	12	12	6
HRT (h) R_2	12	12	12	12	6
Ethanol (g COD/L)	1.0	1.0	1.0	-	0.5
Textile effluent (%)	-	50	100	100	100
AQDS (μM) R_1	-	-	100	100	100
Reactor R_1 was supplemented with AQDS in some periods, and R_2 was AQDS-free during the whole experiment.					

Analyses

Colour was usually analysed three times a week and determined spectrophotometrically (Thermo – Nicolet Evolution 100) by using a single wavelength method (Bisschops and Spanjers, 2003; Hao *et al*., 2000) based on Dos Santos *et al*. (2005a,c) and Somasiri *et al*. (2008) as follows: influent and effluent samples were centrifuged for 2 min at 13,000 rpm (Eppendorf – Mini Spin) and their absorbance was read at the influent sample wavelength of maximum absorbance ($\lambda_{máx,inf}$). However, since the wastewater composition was variable, the $\lambda_{máx,inf}$ value was not fixed. Hence, a visible range (400-700 nm) scan was performed for each influent sample to determine the $\lambda_{máx,inf}$, which usually ranged between 510 and 580 nm.

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

Statistical Methods

The SigmaStat 3.5 computer program was used for the statistical analysis of the data, applying the Mann-Whitney Rank Sum test, a non-parametric procedure that does not require a specific data distribution, to compare the performance of both reactors. The results of the tests were evaluated according to the p-value. If $p \leq 0.050$, the null hypothesis is rejected, i.e., the data groups are considered to be statistically different.

RESULTS AND DISCUSSION

Figure 2 shows the colour removal achieved by the reactors R_1 and R_2 during the experimental periods II to V and Table 2 presents their operational performance during the whole experiment. The operational parameters such as: pH, bicarbonate alkalinity (BA), VFA/TA ratio etc., show that the reactors were stable during the whole experiment and the following discussion will be focused on colour removal.

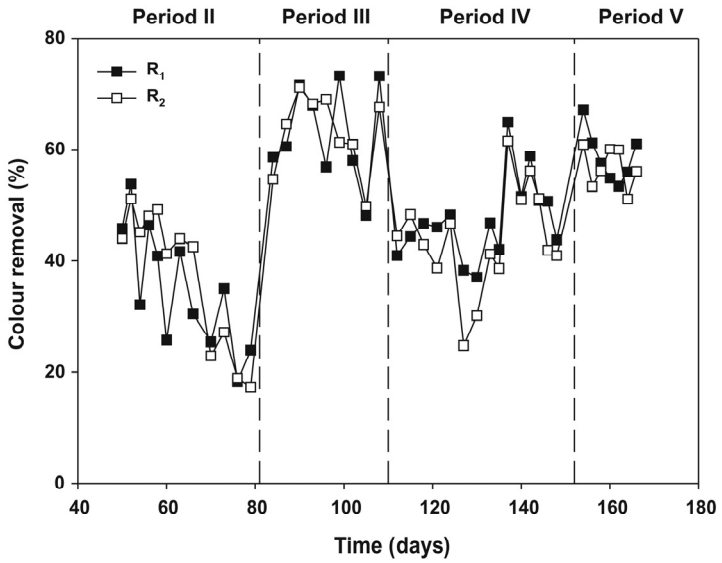

Figure 2: Colour removal performance of the reactors during the periods II to V

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

Operational performance					
Period	**I**	**II**	**III**	**IV**	**V**
Number of samples	**13**	**12**	**9**	**15**	**7**
Total OLR (kg COD/m^3·day)	2.6 ± 0.4	2.6 ± 0.4	3.3 6 ± 0.3	1.6 ± 0.2	2.5 ± 0.2
Influent absorbance (AU) R_1	-	0.272 ± 0.093	0.634 ± 0.124	0.542 ± 0.174	0.717 ± 0.101
Effluent absorbance (AU) R_1	-	0.171 ± 0.057	0.230 ± 0.057	0.275 ± 0.058	0.294 ± 0.038
Colour removal (%) R_1	-	35.8 ± 13.1	63.1 ± 8.8	47.4 ± 7.4	58.7 ± 4.7
Influent absorbance (AU) R_2	-	0.270 ± 0.087	0.672 ± 0.131	0.537 ± 0.180	0.748 ± 0.108
Effluent absorbance (AU) R_2	-	0.157 ± 0.030	0.247 ± 0.059	0.288 ± 0.060	0.321 ± 0.034
Colour removal (%) R_2	-	38.4 ± 12.4	63.1 ± 7.1	43.9 ± 9.3	56.8 ± 3.7
COD removal (%) R_1	78.9 ± 10.1	73.8 ± 4.9	58.8 ± 9.8	35.3 ± 9.6	32.5 ± 4.5
COD removal (%) R_2	86.6 ± 7.1	79.1 ± 3.9	59.1 ± 7.7	36.1 ± 8.9	31.2 ± 4.2
pH R_1	7.3 ± 0.4	7.7 ± 0.3	7.7 ± 0.1	7.5 ± 0.7	8.3 ± 0.6
pH R_2	7.3 ± 0.4	7.7 ± 0.1	7.7 ± 0.1	7.6 ± 0.7	8.3 ± 0.5
BA (mg $CaCO_3$/L) R_1	569 ± 21	740 ± 65	748 ± 5	1003 ± 131	980 ± 174
BA (mg $CaCO_3$/L) R_2	558 ± 22	701 ± 132	709 ± 10	988 ± 131	1027 ± 273
VFA (mg HAc/L) R_1	172 ± 138	210 ± 55	200 ± 25	283 ± 153	297 ± 96
VFA (mg HAc/L) R_2	173 ± 125	199 ± 28	192 ± 16	265 ± 161	278 ± 88
VFA/TA R_1	0.27 ± 0.20	0.26 ± 0.09	0.24 ± 0.03	0.24 ± 0.11	0.26 ± 0.05
VFA/TA R_2	0.27 ± 0.19	0.26 ± 0.08	0.24 ± 0.02	0.23 ± 0.12	0.24 ± 0.03

OLR, organic loading rate; COD, chemical oxygen demand; AU, absorbance unit; BA, bicarbonate alkalinity; VFA, volatile fatty acids.Reactor R_1 was supplemented with AQDS in some periods, and R_2 was AQDS-free during the whole experiment.

After the acclimatization period (period I), the influent was diluted to 50% with distilled water in order to prevent possible toxicity problems (period II). Low average colour removal efficiencies (36-38%) were reached in both reactors in period II, probably due to the low influent absorbance (Table 2). No significant difference between decolourisation efficiencies was observed (p = 0.544). In period III, the reactors were fed with undiluted textile wastewater and R_1 was supplemented with 100 μM AQDS. In this period, higher decolourisation efficiencies were obtained in both reactors since there was an increase in influent absorbance. However, the impact of the redox mediator AQDS on colour removal was not evident since the average efficiency of both reactors was identical (p = 1.000).

Concerning the redox mediator application, these results with a real textile wastewater contradict several previous experiments with synthetic dye-containing wastewaters (Cervantes *et al*., 2001; Dos

Santos *et al*., 2005b; Van der Zee *et al*., 2001). For instance, Cervantes *et al*. (2001) observed during azo dye Acid Orange 7 removal using a UASB reactor (HRT = 6 h) that, in the absence of AQDS, the average efficieny was around 86%, which increased to approximately 99% with the addition of only 30 μM AQDS to the system. Additionally, Dos Santos *et al*. (2005b), who utilized expanded granular sludge bed (EGSB) reactors (HRT = 10 h) to decolourise the azo dye Reactive Red 2, verified that a reactor supplemented with AQDS (25 μM) achieved 88% decolourisation, while the AQDS- free reactor only reached 56%. Van der Zee *et al*. (2001), also in an experiment with a UASB reactor (HRT = 6 h), observed that Reactive Red 2 colour removal efficiencies increased from about 25 to 98% with a gradual increase in AQDS concentration (from 0 to 155 μM). Finally, Silva *et al*. (2011), using an UASB reator opetared at a HRT of 12 h and inoculated with the same sludge source used in the present investigation, found that the Reactive Red 2 (200 mg/L) colour removal increased from 23 to 85% with the addition of only 25 μM AQDS to the system.

However, the impact of redox mediators is not always huge: sometimes the accelerating effect is small, sometimes absent, and in rare cases even adverse since this impact reflects the differences between the systems investigated, the 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 the azo dye Reactive Red 2 (from 20 to 80 mg/L). The authors attributed this result to the low dye concentration tested associated with the high biomass concentration in the reactor, which may have caused the decolourisation reactions to follow zero-order kinetics, therefore masking the AQDS effect. Additionally, for non-recalcitrant dyes such as Congo Red (CR), which has a linear molecular 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, did not result in very evident colour removal, even when very high CR concentrations were employed (> 800 mg/L) (Costa *et al*., 2010).

In contrast to the experiments with synthetic textile wastewaters, Dos Santos *et al*. (2005c) verified that the impact of the redox mediator AQDS (25 μM) on decolourisation rates of a real textile wastewater was not evident. They used a mesophilic EGSB reactor (HRT = 10 h), supplemented with 2.5 g COD/L glucose-VFA mixture (1:3) and the textile wastewater (Ronse, Belgium) was composed of rinsing water and a concentrated solution from the dyebath (variable ratio). Their main hypothesis was that the wastewater was most likely composed of a larger amount of easily reducible dyes and a smaller amount of recalcitrant azo dyes. Thus, the effect of AQDS was probably masked by the wastewater composition.

Apparently, there is no other literature reporting continuous-flow experiments with real textile wastewater and a redox mediator to extend the discussion and conclude if indeed this small effect of redox mediator on colour removal is commom or not.

In period IV, the effect of the external electron donor depletion (ethanol) on colour removal was verified. It was found that the colour removal efficiencies decreased, evidencing that an additional amount of substrate has a positive impact on the decolourisation process. In contrast to the previous period, the average efficiency in R_1 was slightly better than in R_2. Nevertheless, there was not a statistically significant difference between the reactors performance ($p = 0.372$), which also indicates that AQDS did not actually enhance colour removal ability.

In period V, the reactors were supplemented again with ethanol (0.5 COD g/L), but their HRT was reduced to 6 h to assess not only the effect of the HRT on the decolourisation efficiencies but also to verify if, in a system with electron transfer limitation, the AQDS impact would be more apparent. Lower colour removal efficiencies were achieved when compared to the values obtained in period III, which could be attributed to the shorter HRT, since the OLR for the external electron donor was kept the same. Again, although the AQDS-free reactor (R_2) reached an average decolourisation slightly lower, it was not significantly different from R_1 ($p = 0.453$). Thus, an AQDS impact on the R_1 decolourisation efficiency was not verified, showing that the electron transfer from ethanol oxidation to the dye could not be accelerated. Probably, because of type of dyes present in the wastewater, presumably being easily reducible dyes (non-recalcitrant azo dyes) (Dos Santos *et al*., 2005c) or belonging to the anthraquinone class, which are electronically stable due to a resonance effect in their cyclic and conjugated structure (Dos Santos *et al*., 2005a), the redox mediator was less effective in transferring electrons to the dyes present in the real textile wastewater. It seems that under anaerobic conditions the effect of redox mediators on colour removal is more evident with recalcitrant azo dyes and efficiency is usually higher with this type of chromophore compared to anthraquinone ones (Boonyakamol *et al*., 2009; Carliell *et al*., 1994; Dos Santos *et al*., 2005a).

It is important to mention that the decolourisation efficiencies reached during the current experiments are much smaller than the values obtained by Somasiri *et al*. (2008) (~95%) in the treatment of a textile wastewater whose average absorbance was only 0.35. Probably, the absence of expressive peaks in the visible spectrum of the wastewater tested in the present work made colour removal evaluation difficult.

CONCLUSIONS

The anaerobic reactors were able to remove reasonably the colour of real textile wastewaters even when operated with a relatively short HRT (6 h), being a good option of pre-treatment for textile effluents. Aditionally, colour removal efficiency was positively influenced not only by the addition of ethanol as external electron donor, but also by the initial absorbance of the wastewater.

Although the applicability of AQDS is widely reported in literature to remarkably enhance colour removal from synthetic dye-containing wastewaters, especially for recalcitrant azo dyes, the same effect was not evident in the present study with a real textile wastewater, as the reactors did not show significant differences in decolourisation capacity. Hence, further experiments with real textile wastewaters, containing several types of dyes (e.g. azo, antraquinone and phthalocyanine) and from different dyeing processes (batch, continuous and semi-continuous dyeing), need to be carried out in order to assure the applicability of redox mediators in real cases.

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