Artigo

EFFECT OF DYE STRUCTURE AND REDOX MEDIATORS ON ANAEROBIC AZO AND ANTHRAQUINONE DYE REDUCTION

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We investigated the biological decolourisation of dyes with different molecular structures. The kinetic constant values (k_1) achieved with azo dye Reactive Red 120 were 7.6 and 10.1 times higher in the presence of RM (redox mediators) AQDS and riboflavin, respectively, than the assays lacking RM. The kinetic constant achieved with the azo dye Congo Red was 42 times higher than that obtained with the anthraquinone dye Reactive Blue 4. The effect of RM on dye reduction was more evident for azo dyes resistant to reductive processes, and ineffective for anthraquinone dyes because of the structural stability of the latter.

Keywords: colour removal; dyes; structure/activity.

INTRODUCTION

A large variety of dyes with different chemical structures are used in industrial applications. Due to the constant development in the industrial textile sector, there is concern regarding the effluents generated because they may contain a large quantity of dyes that has not fixed to fibres during the finishing step.[1,2]

The release of dye-coloured wastewaters into surface water is undesirable not only for aesthetic reasons and the dyes' impact on aquatic plant photosynthesis and fish mortality, but also because many of these dyes are toxic and carcinogenic.[3-5]

Dyes can be classified according to their chemical structure in terms of chromophore groups, which are responsible for the different dye colours produced. Azo dyes represent the most important dye class used in the textile industry. They are characterized by the presence of one or more azo chromophores (N=N) and bonds between two or more aromatic rings. Anthraquinone dyes constitute the second most important class of textile dyes, which have the chromophore groups, =C=O and =C=C=, forming an anthraquinone complex.[5,6]

Dye characteristics such as resistance to biodegradation, toxicity and resistance to reductive processes, are related to the chemical structure of each dye. As such, due to the wide variety of dyes available in the market and present in textile and other industrial wastewaters, which have different chemical structures, colour removal to be achieved in wastewater treatment plants remains highly complex, because the decolourisation rates are extremely dependent upon the type of dye.[7,8]

Methods currently used to treat textile wastewaters have technical and economical limitations. 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. On the other hand, biological treatment of dyed-waters can be cheaper than physico-chemical treatment.[9-11]

The decolourisation of dyes under anaerobic conditions is based on electrons transfer from a substrate (electron donor oxidized by the microorganisms) to the dye (final electron acceptor), thus promoting the reductive cleavage of the dye chromophore.[5]

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The anaerobic process to remove dye is insufficient to mineralize the degradation products of dyes, so it is necessary a post-treatment for anaerobic effluent. Some researchers have observed that the products of dye degradation, such as aromatic amines, can be chemical compounds that are more toxic than the azo dyes themselves, being characterized by carcinogenic and mutagenic influences. Post-treatment options for these effluents include aerobic biological systems (such as activated sludge) and non-biological processes, such as coagulation-flocculation, activated carbon and AOP (advanced oxidation processes).[7,10,11]

Previous studies have shown the efficiency of anaerobic biological treatment in colour removal of dye-containing wastewaters as well as catalytic effect of redox mediator compounds, such as antraquinone-2,6--disulfonate (AQDS) and vitamin B2 (riboflavin), to speed up the colour removal process signalling good prospects for the application of anaerobic technology even for recalcitrant dyes.[12-15] However, some chemical aspects mainly related to the dye molecule structure, electrons transfer and the presence of redox mediators, are not yet fully understood.

The aim of the present paper was to perform an analysis of the structure/activity relationship and effect of redox mediators on anaerobic azo and anthraquinone dye reduction.

EXPERIMENTAL

Chemicals

The azo dyes Reactive Red 2 (RR2) (Procion Red MX-5B, ~ 50% purity, Aldrich Chemical Company), Congo Red (CR) (analytical grade, Vetec), Reactive Black 5 (RB5) (55% purity, Sigma-Aldrich), Reactive Orange 16 (RO16) (50% purity, Sigma-Aldrich) and Reactive Red 120 (RR120) (60% purity, Sigma-Aldrich), as well as the anthraquinone dyes Reactive Blue 4 (RB4) (35% purity, Sigma-Aldrich) and Remazol Brilliant Blue R (RBBR) (50% purity, Sigma-Aldrich) at an concentration of 0.3 mmol L^{-1} were used in the experiments. The molecular structures of these dyes are depicted in Figures 1 and 2.

The compounds AQDS (~ 98% purity, Aldrich Chemical Company) and riboflavin (98% purity, Sigma-Aldrich) at an concentration of 50 μmol L^{-1} were tested as redox mediators.

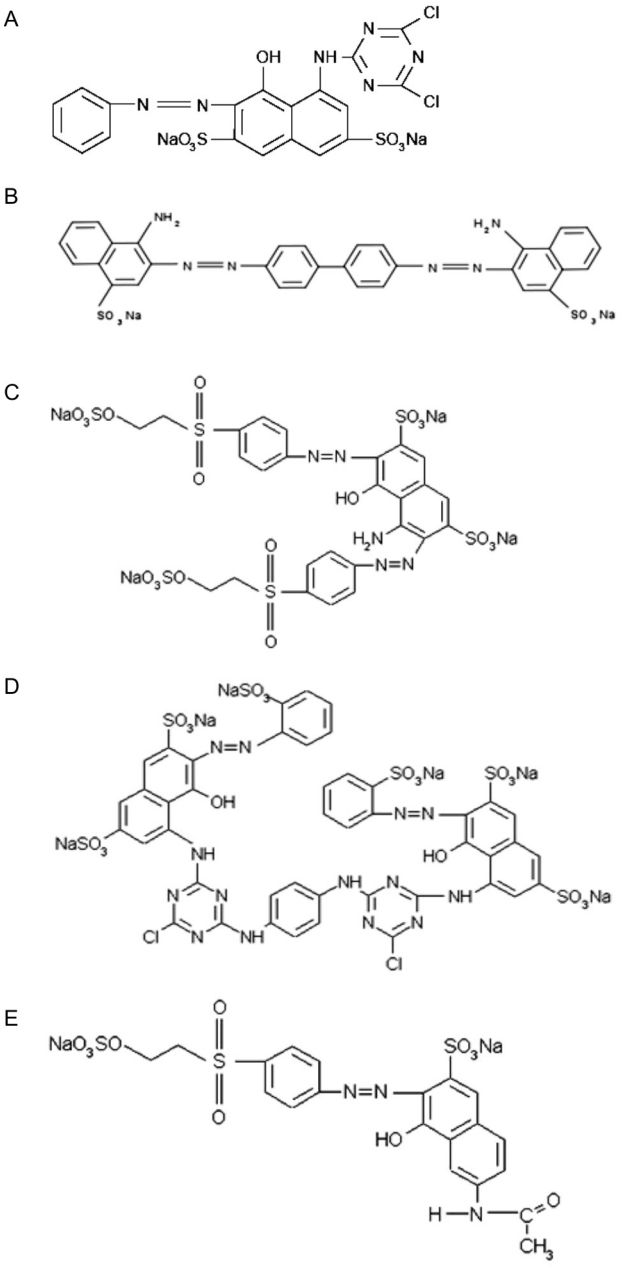

Figure 1. Molecular structure of the azo dyes Reactive Red 2 (A), Congo Red (B), Reactive Black 5 (C), Reactive Red 120 (D) and Reactive Orange 16 (E)

The compound ethanol (CH_3CH_2OH) (99.8% purity, Dinâmica, Brazil) at an concentration of 1.5 g L^{-1} COD (chemical oxygen demand) was used as an electron donor substrate, because previous research demonstrated that this compound is a good electron donor for dye reduction.[5,15]

Sludge source and basal medium

The anaerobic consortium was a sludge collected from an upflow anaerobic sludge blanket (UASB) reactor placed in a brewery (Fortaleza, Ceará, Brazil).

The basal medium used consisted of (mg L^{-1}): NH_4Cl (280), K_2HPO_4 (250), $MgSO_4 \cdot 7H_2O$ (100) and $CaCl_2 \cdot 2H_2O$ (10) and 1 mL L^{-1} of trace elements containing (mg L^{-1}): 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), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (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) and 1 µL L^{-1} of

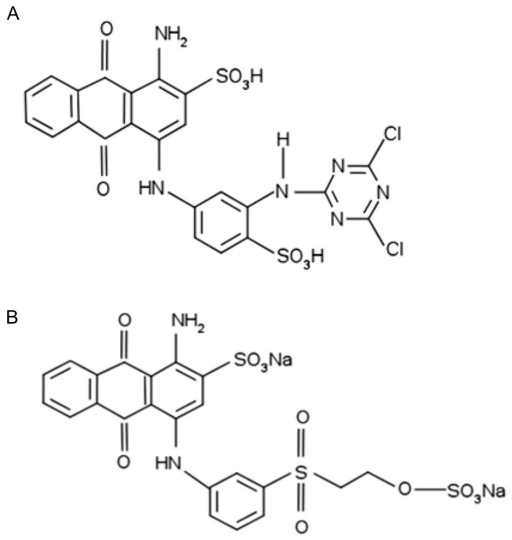

Figure 2. Molecular structure of the anthraquinone dyes Reactive Blue 4 (A) and Remazol Brilliant Blue R (B)

HCl 36% (m/m). The medium was buffered with 2.5 g L^{-1} of sodium bicarbonate to keep the pH around 7.1.

Batch experiments

The non-adapted sludge was transferred to 117 mL-batch assays, with 50 mL of the basal medium described earlier, giving a sludge concentration of 1.5 g L^{-1} VSS (volatile suspended solids).

Batch bottles were sealed with butyl rubber stoppers and aluminium crimp caps, and the headspace was purged with N_2/CO_2 (70%:30%) for 1 min. Subsequently, ethanol, dyes and redox mediators were added to the bottles which were then incubated in a shaker (Tecnal TE-140) at 150 rpm. The experiments were conducted in duplicate at a room temperature was 28 ± 2 °C.

Analyses

A spectrophotometric scan was perfomed on the solution of each dye to verify the wavelength of greatest absorbance (Table 1). The color removal was determined photometrically at a wavelength of greatest absorbance of each dye on a Thermo - Nicolet Evolution 100 spectrophotometer.

Table 1. Values for the wavelengths of greatest absorbance of each dye

Dyes	Wavelength (nm)
Reactive Red 2	539
Congo Red	486
Reactive Black 5	579
Reactive Orange 16	492
Reactive Red 120	514
Reactive Blue 4	599
Remazol Brilliant Blue R	594

Samples were diluted in a phosphate buffer and placed into Eppendorf tubes. In order to avoid turbidity interference, samples were centrifuged at 13,000 rpm for 2 min.

Sample collection was done considering visual changes in the bottles in terms of colour loss. However, most of the time samples were collected at the same time.

The linear potentiodynamic polarization (LPP) technique was used to measure the redox potentials of electron donor, redox mediators and dyes. A conventional electrochemical cell of three electrodes was used. Platinum was the counter electrode and working electrode, with a superficial area of approximately 2 cm² and saturated calomel was the reference electrode. A potentiostat/galvanostat was used (Autolab, model PGSTAT 30), and the scan rate was 5 mV s^{-1}, in all electrochemical analyses. Analyses were done at room temperature without stirring. Samples were diluted with ultra-pure water collected in a Milli-Q system (Millipore Corporation) and analyzed at the same concentration as the batch experiments.

RESULTS AND DISCUSSION

Structure-activity relationship on dye reduction in assays free of and supplemented with redox mediators

The consumption of 1 mol of O_2 represents a reaction with 4 mols of electrons (Equation 1); if 1 mol of O_2 represents 32 g of COD, 1 mol of electrons corresponds to 8 g of COD. For a monoazo dye, theoretically, 4 electrons are necessary to reduce one molecule. Therefore, to reduce 0.3 mmol L^{-1} of dye, 1.2 mmol L^{-1} of electrons (9.6 mg L^{-1} COD) are necessary; for a diazo dye 8 electrons are necessary, in other words 2.4 mmol L^{-1} of electrons (19.2 mg L^{-1} COD).

$$2H_2O \rightarrow O_2 + 2H_2 + 4e^- \quad (1)$$

For the anthraquinone dyes, 4 electrons are required for the chromophore cleavage, equivalent to 9.6 mg L^{-1} COD for a dye concentration of 0.3 mmol L^{-1}.

In the present investigation, a concentration of 0.3 mmol L^{-1} was used for all dyes tested, and 1.5 g L^{-1} COD was the electron donor substrate concentration. Therefore, an abundance of electrons existed in the system, and the results of decolourisation are wholly related to the different chemical structures of the azo and anthraquinone dyes tested.

The first-order kinetic constants achieved for anaerobic azo and anthraquinone dye reduction in systems free of and supplemented with redox mediators are shown in Table 2.

Azo dye Congo Red showed a high decolourisation rate, probably due to its linear structure compared to the other dyes tested, which decreases the steric hindrance effect (Figures 1 and 2). Therefore, it is easier for a molecule of this type of azo dye to receive the electrons required for dye reduction compared to the azo dyes Reactive Black 5 and Reactive Orange 16, for example, which have a conjugated structure and subsequent steric hindrance effect.

In the absence of redox mediators, Congo Red´s kinetic constant (k_1 = 3.8 day^{-1}) was about 15.2 and 7 times higher than the values found for Reactive Red 120 (k_1 = 0.25 day^{-1}) and Reactive Red 2 (k_1 = 0.54 day^{-1}), respectively. The low colour removal rates achieved with these dyes in comparison to the other azo dyes tested is likely due to the high recalcitrance for reductive processes as a result of the competition for electrons between nitrogen atoms from the triazine group and nitrogen from the azo linkage.

Analyzing the k_1 values reported in van der Zee *et al.*[9] achieved during anaerobic azo dye reduction by mesophilic sludge in the absence of redox mediators, it is possible to conclude that dyes containing the triazine group in their molecular structure (Reactive Red 2, Reactive Red 4, Reactive Orange 14 and Reactive Yellow 2) presented low decolourisation rates, compared to the other dyes tested. Additionally, it was also possible to verify a structure/activity relationship. For example, the dye Direct Red 81 (k_1 = 7.8 day^{-1}) had a kinetic constant 3.7 times higher than the value found for Reactive Orange 16. The chemical structure of Direct Yellow 81 is linear, which probably reduced the steric hindrance effect, as opposed to Reactive Orange 16, which has a large molecular volume, making its reduction difficult.

In the current experiment, anthraquinone dyes presented the lowest decolourisation rates amongst the dyes tested (Table 2). Anthraquinone groups have a resonance effect on their cyclic and conjugated structure, which stabilizes the chemical structure of the dye.[16]

The anthraquinone dye Reactive Blue 4 was the most recalcitrant dye for anaerobic decolourisation, presenting the lowest kinetic constant (k_1 = 0.09 day^{-1}). The kinetic constant achieved with the azo dye Congo Red was 42 times higher than that obtained with the anthraquinone dye Reactive Blue 4. Analysis of the chemical structure of anthraquinone dye Reactive Blue 4 reveals that the recalcitrance nature can be related with both the presence of the triazine group in its structure and the anthraquinone chromophore.

Interestingly, the anthraquinone dye Reactive Brilliant Blue R presented k_1 values higher than some azo dyes considered recalcitrant, even though the values were still very low (k_1 = 0.81 day^{-1}). This behavior was probably because the molecular structure of Reactive Brilliant Blue R does not probably a high molecular volume compared to the azo dye Reactive Black 5, or does it have a triazine group, as do the azo dyes Reactive Red 2 and Reactive Red 120.

According to Lee and Pavlostathis,[8] repetitive feeding of anthraquinone dyes in anaerobic bioreactors may cause a toxic effect on methanogens, and a subsequent decrease in dye decolourisation capacity and electron donor removal.

AQDS addition enhanced azo dye reduction in all cases (Table 2). For instance, the k_1-value achieved with Reactive Red 2 and Reactive Red 120 were 3.5 and 7.6 times higher than the assays lacking redox mediators, respectively. Interestingly, the highest AQDS impact was found with the most recalcitrant azo dyes (based on the molecular structure analysis). However, with the anthraquinone dyes Reactive Blue 4 and Reactive Brilliant Blue R, AQDS addition did not ca-

Table 2. Structure/activity relationship for assays free and supplemented with redox mediators

Dye	Molecular weight (g mol^{-1})	k_1 (day^{-1}) Without redox mediators	k_1 (day^{-1}) With AQDS (50 µmol L^{-1})	k_1 (day^{-1}) With Riboflavin (50 µmol L^{-1})
Reactive Red 2	615.3	0.54	1.88	2.83
Congo Red	697.0	3.80	5.12	5.12
Reactive Black 5	991.8	0.59	1.85	4.32
Reactive Orange 16	617.5	2.70	3.50	5.23
Reactive Red 120	1469.0	0.25	1.90	2.52
Reactive Blue 4	637.4	0.09	0.15	0.22
Remazol Brilliant Blue R	626.6	0.81	0.33	0.40

talyse dye reduction. On the contrary, the Reactive Brilliant Blue R decolourisation rate in presence of AQDS was lower than the control lacking this compound. In this case, the electrons were probably used to reduce AQDS and could not be further transferred to RBBR. Similar results in terms of low decolourisation rate with anthraquinone dyes were reported by Dos Santos *et al*.[5] while testing the dye Reactive Blue 5 with anaerobic granular sludge.

In theory, redox mediators are more effective for catalysing azo dye reduction because of their azo chromophore nature, which is electronically unstable and has the capacity to receive electrons from the reduced form of the mediator. However, anthraquinone dyes are electronically stable, and therefore, the redox mediator, following reduction will be less efficient or ineffective for the transfer electrons to the dye.[17]

Riboflavin addition enhanced azo dye reduction in all cases and, compared to AQDS, was a more suitable redox mediator (Table 2). The k_1-values achieved with Reactive Red 2 and Reactive Red 120 were 5.2 and 10.1 times higher than the assays lacking redox mediators, respectively. The riboflavin effect was also more evident with the recalcitrant azo dyes.

For the azo dye Congo Red, the redox mediators AQDS and riboflavin had the same impact on dye reduction. This finding can be attributed to the dye molecule, which was easily reduced, thus masking the effect of these catalytic compounds on decolourisation rates.

Riboflavin proved unable to significantly enhance anthraquinone dye reduction rates. For Reactive Blue 4, the k_1-value was increased 2.4-fold compared to controls lacking redox mediators, but the value (0.22 day^{-1}) remained very low compared to the other kinetic constants. For Reactive Brilliant Blue R, riboflavin addition decreased the dye reduction rate, as was found with AQDS (Table 2).

In the present study, no correlation between dye reduction rate and molecular weight (MW) in the absence of redox mediators was found. As shown in Table 2, the kinetic constant of Reactive Black 5 (MW: 991.82 g mol^{-1}) was 1.8 times higher than the value found for Reactive Red 2 (MW: 615.34 g mol^{-1}). Comparing the two anthraquinone dyes, the k_1-value of Reactive Brilliant Blue R (k_1 = 0.81 day^{-1}) was 9 times higher than the value of Reactive Blue 4 (k_1 = 0.09 day^{-1}), although the molecular weights of both dyes were very similar. Comparing dyes with similar molecular weights, the decolourisation rate for the azo dye Congo Red (MW: 697.0 g mol^{-1}) was 42 times higher than the rate found for the anthraquinone dye Reactive Blue 4 (MW: 637.4 g mol^{-1}).

The redox mediator effect is not related to dye molecular weight but rather to dye recalcitrance to reductive processes. For instance, data shown in Table 2 indicate that AQDS addition enhanced the RR2 reduction rate (MW: 615.34 g mol^{-1}) 3.5 fold compared to the control (redox mediator free), while for CR (MW: 696.98 g mol^{-1}) this increment was only 1.3 fold.

In the absence of external redox mediators, van der Zee *et al.*[9] also failed to find any correlation between the kinetic constant k_1 and molecular weight. The main experiment conclusion was that dye reduction likely occurred outside the cell. For instance, these authors found that the azo dye Direct Red 79 (MW: 1049 g mol^{-1}) reduced much faster (k_1 = 16.6 day^{-1}) than the azo dye Mordant Orange 1 (MW: 287 g mol^{-1}, k_1 = 1.74 day^{-1}) under the same experimental conditions. However, no analysis was done based on the different dye chemical structures.

Other studies have shown the influence of structural differences on dye reduction rates. For instance, Brás *et al*.[18] tested the monoazo Acid Orange 7 and the diazo Direct Red 254, and observed that structural differences between dye molecules probably influenced the reduction rates by anaerobic sludge. Diazo Direct Red 254 achieved higher reduction rates compared to monoazo Acid Orange 7. According to these authors, azo bond location was an important factor to be considered in azo dye reduction, together with sulfonic group location, which influenced steric hindrance.

Isik and Sponza[19] studied anaerobic reduction of the dyes Direct Brown 2 and Reactive Black 5, and found that the k_1-value was considerably higher for Reactive Black 5. This finding was attributed to the nature of the Direct Brown 2 auxochromes and to the fact that the aromatic amines produced could be toxic to microorganisms.

Application of linear potentiodynamic polarization (LPP) in structure/activity analyses of dye reduction

In order to explain the differences between the redox mediators we tested, AQDS and riboflavin redox potentials were compared to the redox values of electron donor and dyes, under similar experimental conditions to the batch tests, by using LPP.

Analysis of oxidation potential values (Table 3) showed that ethanol was preferentially oxidized compared to the other species present, given its oxidation potential (E_{ox} = 0.249 V) was the highest among the samples, thereby confirming ethanol as a good electron donor.

Table 3. Values of the oxidation potentials measured by LPP

Sample	Sample characteristics	E_{ox} (V *vs* SCE)
Ethanol	Electron donor Substrate	0.249
AQDS	Redox mediator	0.112
Riboflavin	Redox mediator	0.153
Reactive Red 2	Dye	0.129
Congo Red	Dye	0.206
Reactive Black 5	Dye	0.205
Reactive Orange 16	Dye	0.201
Reactive Red 120	Dye	0.217
Reactive Blue 4	Dye	0.171
Remazol Brilliant Blue R	Dye	0.177

Ethanol is initially oxidized and subsequently transfers electrons to the mediator. After mediator reduction, the electrons are transferred to the dye to promote chromophore reduction. AQDS and riboflavin have the ability to work as redox mediators because they have redox potential between redox values of ethanol oxidation and dyes reduction. Riboflavin redox potential (E_{redox} = ± 0.153 V) indicates a better electron transfer capacity compared to AQDS (E_{redox} = ± 0.112 V) (Table 3).

Furthermore, biomass affinity and electrochemical interaction between dye and redox mediator also play a role in dye reduction. Field and Brady[20] observed high affinity of an anaerobic granular sludge for riboflavin during Mordant Yellow 10 reduction. Additionally, Rau *et al.*[21] found that the catalytic effect of different redox mediators on azo dye reduction varied significantly with the pure cultures tested.

The dyes tested have a narrow redox potential range of between 0.129 and 0.217 V (Table 3). A correlation between redox potential and dye reduction rates was observed. It seems that the greater the similarity of redox potencials of dye and redox mediator, the faster the dye reduction, since electron transfer is facilitated due to the low potential difference.

Such behaviour explains the better catalytic properties of riboflavin as compared to AQDS. However, dye reduction rate is not only determined by redox potential, but also by other factors such as chemical structure, environmental conditions and anaerobic sludge affinity and concentration.[10,21,22]

Final decolourisation of azo and anthraquinone dyes

Amongst the azo dyes tested, Congo Red had the highest values for final decolourisation (97.5%), confirming that it was the least recalcitrant dye to reductive processes. For dyes that contained the triazine group, which exhibited low rates of colour removal, the final decolourisation was satisfactory, with 87.9% for Reactive Red 2 and 91.6% for Reactive Red 120 (Figure 3).

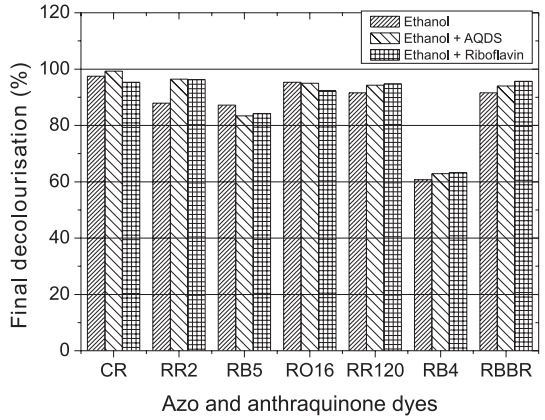

Figure 3. *Final decolourisation results of azo and anthraquinone dyes achieved in assays free and supplemented with the redox mediators AQDS and Riboflavin, with ethanol as electron donor (1.5 g L^{-1} COD)*

In the experiments performed with either AQDS or riboflavin, colour removal efficiency was generally higher than in the redox mediator-free control. For instance, when Reactive Red 2 was tested, colour removal efficiency was 96.4% for the AQDS-supplemented bottles, i.e. 8.5% higher than the redox mediator-free control. For Reactive Red 120, the difference in relation to the control was only 2.7 and 3.2% for the bottles containing AQDS and riboflavin, respectively. Because redox mediators catalyse dye reduction, its effect is better observed by dye reduction rates than by final decolourisation (Figure 3 and Table 2).

Regarding the anthraquinone dyes tested, high colour removal efficiency was only achieved for Reactive Brilliant Blue R (91.6%). For Reactive Blue 4, decolourisation was only 60.7%, being the most recalcitrant dye tested in terms of final colour removal (Figure 3) and reduction rates (Table 2).

In a study involving UASB reactors, Panswad and Luangdilok[23] reported 64 and 63% colour removal for the anthraquinone dyes Reactive Blue 19 (20 mg L^{-1}) and Reactive Blue 5 (20 mg L^{-1}), respectively, using a mixture of glucose and acetic acid (1000 mg L^{-1} COD) as the electron donor.

Therefore, anthraquinone dye removal under anaerobic conditions represents a concern, and therefore further research using biological and non-biological methods should conducted.

CONCLUSIONS

Congo Red was the least recalcitrant dye tested, probably due to its linear structure compared to the other dyes tested, which decreased the steric hindrance effect. Dyes that contained triazine in their structure were more recalcitrant due to the high stability this group confers to dye molecules rendering azo link cleavage more difficult. The addition of redox mediators, however, considerably facilitated azo cleavage.

Anthraquinone dye reduction by anaerobic sludge proved very difficult and redox mediators did not catalyse the reaction, probably due to structural stability, high molecular volume and steric hindrance effect.

Redox mediator addition enhanced azo dye reduction, and its effect was more evident with the recalcitrant dyes. Riboflavin was a much better redox mediator for dye reduction compared to AQDS, increasing the k_1-value by up to one order of magnitude compared to the assays lacking redox mediators.

In the absence or presence of redox mediators, no correlation between dye reduction rate and molecular weight was found; the redox mediator effect is related to dye recalcitrance to reductive processes.

The LPP method and structure/activity relationship analysis are very important to help further understanding of dye reduction mechanisms under anaerobic conditions in systems supplemented by, and free of, redox mediators.

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