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Sequential Anaerobic/Aerobic Treatment of Dye-Containing Wastewaters: Colour and COD Removals, and Ecotoxicity Tests

Marcos Erick Rodrigues da Silva · Paulo Igor Milen Firmino · Márcia Rodrigues de Sousa · André Bezerra dos Santos

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Abstract Colour and COD removals of the azo dyes Congo Red (CR) and Reactive Black 5 (RB5) were individually evaluated in a sequential anaerobic/aerobic treatment system. Additionally, dye toxicity was assessed by using acute ecotoxicity tests with *Daphnia magna* as the indicator-organism. The anaerobic reactor was operated at approximately 27 °C and with hydraulic retention times of 12 and 24 h. The aerobic reactor was operated in batch mode with a total cycle of 24 h. During anaerobic step, high colour removals were obtained, 96.3% for CR (400 mg/L) and 75% for RB5 (200 mg/L). During the aerobic phase, COD effluent was considerably reduced, with an average removal efficiency of 52% for CR and 85% for RB5, which resulted in an overall COD removal of 88% for both dyes. Ecotoxicity tests with CR revealed that the anaerobic effluent presented a higher toxicity compared with the influent, and an aerobic post-treatment was not efficient in reducing toxicity. However, the results with RB5 showed that both anaerobic and aerobic steps could decrease dye toxicity, especially the aerobic phase, which removed completely the toxicity in *D. magna*. Therefore, the anaerobic/aerobic treatment is not always effective in detoxifying dye-containing wastewaters, sometimes even increasing dye toxicity.

Keywords Anaerobic/aerobic treatment · Azo dyes · Reductive decolourisation · Toxicity · *Daphnia magna*

M. E. R. da Silva · P. I. M. Firmino · M. R. de Sousa · A. B. dos Santos
Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil

M. E. R. da Silva
Federal Institute of Education, Science and Technology of Ceará, Campus Sobral, Sobral, Ceará, Brazil

A. B. dos Santos (✉)
Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Campus do Pici, Bloco 713. Pici, CEP: 60455-900 Fortaleza, Ceará, Brazil
e-mail: andre23@ufc.br

Introduction

Although there was a marked decline in all sectors of manufactured goods in 2009, the textile industry still represents an important economic sector worldwide, being responsible for 1.7% of world exportation in that year, which corresponded to the amount of US$ 211 billions [1]. Thus, with increased demand for textile products in the last years (3% increase in exports in 2000-2009) [1], a proportional wastewater generation increase was observed, through which a large amount of chemicals is released into surface waters, representing a serious environmental problem and a public health concern [2, 3].

One of the main characteristics of textile wastewaters is that they are highly coloured, mainly due to the dyes applied in dyeing step. Depending on dye class, the percentage of dye that remains unfixed to the fibre during dyeing process varies from 5% up to 50% on a weight base [2]. Although recent statistics on global production of dyes are not readily available [2], it is estimated that over 10,000 tons of dyes are produced annually worldwide, amongst which azo dyes are the most employed at industrial scale (>50%), followed by anthraquinone and phthalocyanine dyes [4].

Amongst the different decolourisation methods, biological treatment has called attention for being economically attractive, usually easy to operate and generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants [5]. However, colour removal by aerobic bacteria, such as those commonly present in activated sludge systems, is normally low [3], which is mainly associated with dye adsorption on the sludge [6, 7]. On the other hand, under anaerobic conditions, effective dye decolourisation can be reached [8–10].

According to van der Zee and Villaverde [11], azo dye biodegradation proceeds in two stages. The first stage involves reductive cleavage of the dyes' azo linkages under anaerobic conditions, resulting in aromatic amines formation, which are generally colourless but potentially hazardous. The second stage involves degradation of the aromatic amines under aerobic conditions since these compounds are usually recalcitrant under anaerobic conditions. Hence, a wastewater treatment process in which anaerobic and aerobic conditions are combined is reported to be the most logical and economical concept for removing azo dyes from wastewater [12], and it has been extensively investigated [11, 13–22].

However, during application of a sequential anaerobic (or microaerophilic)/aerobic treatment, it is very important to assess both aromatic amine mineralisation as well as the removal of other toxic compounds in textile wastewaters. An indirect form of evaluation of these by-products' (aromatic amines) mineralisation is either following chemical oxygen demand (COD) reduction or applying a toxicity evaluation [13]. Therefore, acute toxicity assays of effluents generated in this sequential treatment is an important tool for assessing mineralisation and detoxification ability of biological processes on textile wastewaters.

Daphnia magna has been evaluated as a good organism to test effluent toxicity in dye-containing wastewaters since toxicity assays with this organism are standardised and reliable [23–27]. However, just few papers on sequential anaerobic (or microaerophilic)/aerobic treatment reported toxicity studies using *D. magna* as the indicator-organism [13, 22, 28, 29].

Hence, the present work aimed to assess the performance of a sequential anaerobic/aerobic system to remove colour, COD and toxicity of synthetic wastewaters containing the azo dyes Congo Red (CR) and Reactive Black 5 (RB5).

Materials and Methods

Synthetic Wastewater

Synthetic wastewater was composed of distilled water, an azo dye, a carbon source (electrons donor), basal medium (nutrients) and a buffer. CR (or Direct Red 28 (DR28)) (analytical grade, Vetec, Brazil) and RB5 (55% purity, Sigma-Aldrich, USA) were individually used (Fig. 1) at the concentrations of 400 and 200 mg/L, respectively. The electron donor compound (1.0 g COD/L) was ethanol (99.8% purity, Dinâmica, Brazil), and the basal medium composition was according to Firmino et al. [30]. To keep 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.

Experimental System

The experimental lab-scale wastewater treatment system consisted of a mesophilic continuous-flow upflow anaerobic sludge blanket (UASB) reactor followed by a mesophilic aerobic sequencing batch reactor (SBR), which operated during 65 and 37 days for the dyes CR and RB5, respectively.

The UASB reactor (working volume of 5.2 L) was 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. The reactor was operated with hydraulic retention times (HRT) of 24 and 12 h during the experiments with CR and RB5, respectively. Influent was stored at 4 °C, and the reactor was operated at room temperature of approximately 27 °C.

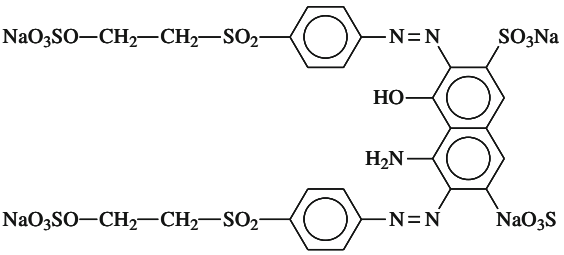
The aerobic SBR (working volume of 3.5 L), which treated the UASB effluent at a room temperature of approximately 27 °C, was inoculated with an aerobic sludge from a brewery mesophilic activated sludge system (Industrial District, Ceará, Brazil) at a final concentration of approximately 2.8 g VSS/L. The SBR was operated at a total cycle of 24 h for both dyes, whose sequencing phases were set as follows—filling (1 h), reaction (22 h), settling (1.5 h) and idle (0.5 h). The oxygen was provided by air compressors, and its liquid phase distribution was uniformly done by porous stones installed at the reactor bottom, which maintained the dissolved oxygen (DO) concentration above 2.6 mg/L.

Ecotoxicity Tests

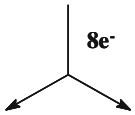
Acute toxicity tests using *D. magna* (Crustacea, Cladocera) as the indicator-organism were carried out according to NBR 12713 [31] in order to assess if anaerobic/aerobic sequential treatment could reduce dye-containing wastewater toxicity.

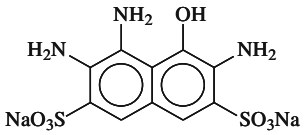
The organisms were cultured in separated lots of 40 individuals in 2 L culture medium (pH 7–8, hardness 175–225 mg/L as $CaCO_3$, DO>7 mg/L) which consisted of (milligrams per liter) $CaCl_2{\cdot}2H_2O$ (235.2), $MgSO_4{\cdot}7H_2O$ (98.64), KCl (4.64) and $NaHCO_3$ (51.84), and 1 mL/L of (milligrams per liter) $MnCl_2{\cdot}4H_2O$ (721), LiCl (612), RbCl (142), $SrCl{\cdot}H_2O$ (304), $CuCl{\cdot}2H_2O$ (33.5), $ZnCl_2$ (26), $CoCl_2{\cdot}6H_2O$ (20), $NaNO_3$ (274), H_3BO_3 (2859.5), NaBr (16), $Na_2MoO_4{\cdot}2H_2O$ (63), NH_4VO_3 (0.575), KI (3.25), Na_2SeO_3 (3.19), Na_2SiO_3 (4293), $Na_2EDTA{\cdot}7H_2O$ (2500), $FeSO_4{\cdot}7H_2O$ (995.5), KH_2PO_4 (143), K_2HPO_4 (184), thiamine hydrochloride (75), cyanocobalamin (1) and biotin D(+) (0.75) [31].

The lots of *D. magna* contained only female organisms since they reproduce via parthenogenesis, which guaranteed that all individuals were clones. They were maintained in a germination chamber (Tecnal–TE401) at a temperature of approximately 20 °C and with a

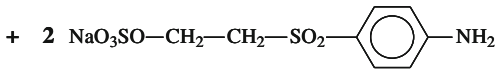


Reactive Black 5 (RB5)

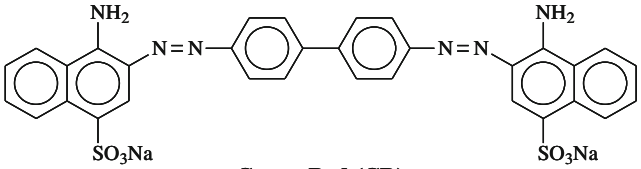




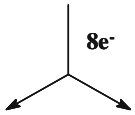
Sodium 1,2,7-triamino-8-hydroxynaphthalene-3,6-disulphonate (TAHNDS)

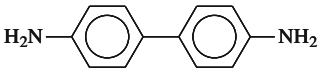


Sodium 2-(4-aminobenzene- sulphonyl)-ethoxi-sulphonate (ABSES)

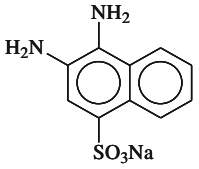


Congo Red (CR)





Benzidine



Sodium 1,2-diaminonaphthalene-4-sulphonate (DANS)

Fig. 1 Chemical structure of the azo dyes CR and RB and their expected aromatic amines produced from complete azo bonds cleavage

photoperiod of 16 h of light and were fed daily with a suspension of the algae *Pseudokirchneriella subcaptata* at a concentration of $4.5\,10^6$ cells/mL per adult organism.

Additionally, in order to evaluate organisms' physiological conditions, sensitivity tests were performed monthly. Potassium dichromate ($K_2Cr_2O_7$) was the reference substance tested at different concentrations (0, 0.125, 0.25, 0.5, 0.8 and 1.0 mg/L). These tests were performed at similar conditions of the toxicity experiments in order to do a control chart.

Acute toxicity tests with samples of CR- and RB5-containing wastewaters (UASB influent and effluent, and SBR effluent), at different concentrations, were conducted with neonates (2–26 h old) of *D. magna* during 48 h in a germination chamber (Tecnal–TE401) at a temperature of 18–22 °C and with a photoperiod of 16 h of light.

For each sample concentration, a minimum number of 21 organisms (divided in triplicate) were added in 50-mL beakers. Then, the number of immobile or dead organisms was counted after 48 h, and the EC_{50} parameter, i.e. concentration that causes 50% death or immobility of the indicator-organism, could be calculated by using the Trimmed Sperman–Karber statistic program, with 95% confidence interval.

Analyses

Colour was usually analysed three times a week and determined photometrically (Thermo–Nicolet Evolution 100) by using a single wavelength method [32]. The absorbance of each dye was read at the wavelength (λ) whose absorbance is maximum, which is 496 and 598 nm for CR and RB5, respectively. 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, total alkalinity (TA) 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 a potentiometric method (Digimed–DM 20) and TA by a titrimetric method, all of them according to Standard Methods for the Examination of Water and Wastewater [33]. VFA were determined using the Kapp titrimetric method [34].

Statistical Methods

SigmaStat 3.5 computer program was used for the statistical analysis of the data, being applied the Mann–Whitney Rank Sum test, a non-parametric procedure which does not require a specific data distribution, to compare the performance of the reactors during the experiments with both dyes. 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 statistically different.

Results and Discussion

Anaerobic Treatment

Colour Removal

The experiment with the dye CR (400 mg/L) revealed that UASB reactor provided high colour removal efficiencies (96.3%), whereas only 75.2% were achieved with the dye RB5

in a lower influent concentration (200 mg/L) (Table 1), i.e. there was a statistical difference between these experimental periods ($p<0.001$). These results showed that RB5 is more recalcitrant than CR since the linear molecule structure of the latter allows easy chromophore reduction even when very high CR concentrations were applied (>800 mg/L) at a shorter HRT (8–12 h) [9, 30]. Additionally, colour removal is more difficult with highly substituted and high molecular weight dyes such as RB5 [28].

COD Removal and Operational Stability

For CR, the UASB reactor showed a good operational stability since low VFA concentrations were detected in the anaerobic effluent (Table 1). The average COD removal obtained was approximately 60%, but residual COD was still considerably high (464.5 mg/L).

Diniz et al. [35] reported that the azo dye CR was toxic to cells of the organism *Desulfovibrio alaskensis* in concentrations higher than 0.5 mM (~350 mg/L). However, no inhibition was observed in the anaerobic reactor of the present work, which agrees with Costa et al. [9], who also did not find any inhibition of CR or its reduced products in terms of substrate (ethanol) oxidation even when their UASB reactors were fed with approximately 850 mg/L of dye. Moreover, Sponza and Işik [22] did not observe any inhibitory effect on the anaerobic sludge of a UASB treating a CR-containing wastewater with a dye concentration as high as 3,200 mg/L. Therefore, it is advantageous to use anaerobic consortia compared with pure cultures because the high microbial diversity in anaerobic consortia helps to decrease toxicity effects and enhance process stability [9].

On the other hand, for RB5, there was an accumulation of VFA in the reactor, i.e. VFA/TA relation varied from 0.5 to 0.7, which is higher than the critical value (0.4) reported by Behling et al. [36]. Hence, these results indicated that, during RB5 treatment, a possible anaerobic microbiota inhibition might have occurred. Additionally, the average COD removal was remarkably low (28.1%), achieving a minimum value of 19.4%, which reinforces the inhibition hypothesis. As a result, the average residual COD was significantly high (863.9 mg/L) (Table 1). Again, it was confirmed that there is a statistical difference ($p<0.001$) in COD removal performance of the reactor in the presence of different dyes.

A possible explanation for RB5 toxicity may be related to its non-hydrolysed supplementation in the bioreactors. For instance, Libra et al. [16] reported that, when partially hydrolysed, RB5 was found to almost completely suppress the methanogenic and sulphate-reducing activity of a bioreactor, whereas no significant inhibition was observed when the reactor treated the fully hydrolysed RB5. Therefore, concerning the toxicity of vinylsulphonic reactive azo dyes, such

Table 1 UASB reactor performance during the treatment of synthetic wastewaters containing the dyes CR and RB5

Dye	CR	RB5
Performance of the reactor		
Colour removal (%)	96.3 (1.5)	75.2 (5.5)
COD removal (%)	59.9 (15.7)	28.1 (7.7)
COD effluent (mg/L)	464.5 (190.9)	863.9 (108.2)
pH	7.1 (0.3)	7.7 (0.1)
TA (mg/L)	248.9 (33.9)	616.0 (39.1)
VFA (mg/L)	78.2 (47.8)	371.4 (70.2)
VFA/TA	0.3 (0.1)	0.6 (0.1)

The standard deviation is shown in parenthesis

as RB5, to anaerobic biomass, hydrolysis of the reactive groups (vinylsulphone) seems to be very important [11].

In contrast, no inhibition was observed by Işik and Sponza [37] in anaerobic batch toxicity tests even at concentrations as high as 1,200 mg/L of non-hydrolysed RB5. Also, Sponza and Işik [20] did not find any problems in COD removal by using a UASB reactor treating a synthetic wastewater containing 100 mg/L of non-hydrolysed RB5 supplemented with glucose (3,000 mg/L COD) unless very high organic loading rates were applied (20–25 kg COD/m^3·day), i.e. average COD removal decreased from 56% (at 4.83 kg COD/m^3·day) to 26.6% (24.6 kg COD/m^3·day) most likely due to the accumulation of intermediate degradation products such as VFA and breakdown products.

Hence, from the presented results, a post-treatment is necessary not only to reduce residual colour and COD from anaerobic effluent but also to mineralise the aromatic amines generated, which are potentially carcinogenic and mutagenic [38, 39].

Aerobic Post-treatment

Colour Removal

Figure 2 shows the influent and effluent absorbance spectra for the synthetic dye-containing wastewaters. In relation to the UASB effluent, a reduction of the peaks at 486 (CR) and 598 nm (RB5) and formation of another peak at 260 nm (UV range) were observed. The absorbance spectrum changes at UV-vis range evidence azo bond cleavages and, consequently, aromatic amines generation [39].

Regarding the overall colour removal performance of the sequential anaerobic/aerobic system, a total CR decolourisation practically occurred only in the UASB reactor, whereas SBR performance was negligible (Fig. 2a). In contrast, for RB5, a colour reduction was observed during aerobic treatment (Fig. 2b). Therefore, the sequential anaerobic/aerobic system achieved an overall average decolourisation of 88.1% for this latter dye. Nevertheless, although SBR has not reduced residual colour of CR, the sequential system was still more efficient for this dye than for RB5 ($p=0.006$).

Sponza and Işik [21], using an anaerobic/aerobic system to treat a synthetic wastewater containing the dye Direct Black 38, whose dye load was near to the applied in this research, observed that most of the decolourisation was due to the anaerobic step. Ong et al. [19] also mentioned that, during application of a sequential anaerobic/aerobic system on Acid Orange 7 azo dye, the anaerobic phase was the main responsible for decolourisation, and, under aerobic conditions, colour removal was negligible.

The absorbance decrease at UV range during aerobic step (Fig. 2) might be evidence of a probable mineralisation of CR and RB5 by-products (aromatic amines). However, some aromatic amines may also react with oxygen via free radical reactions (autoxidation), resulting in the formation of undesirable coloured oligomers and polymers with low solubility, which are easily removed from the water phase [12]. The autoxidation phenomenon was specifically observed for CR in the present investigation since colour development—different from the original hue—was visually detected after the aerobic post-treatment, which was confirmed by some slight changes in the visible range of the absorbance spectrum (data not shown).

Several studies on azo dye reduction indicate that most of the aromatic amines produced were removed in aerobic phase [11, 39]. However, some aromatic amines cannot be removed aerobically, especially the sulphonated ones, which are difficult to degrade since the hydrophilic nature of the sulphonate group obstructs membrane transport [40], such as the

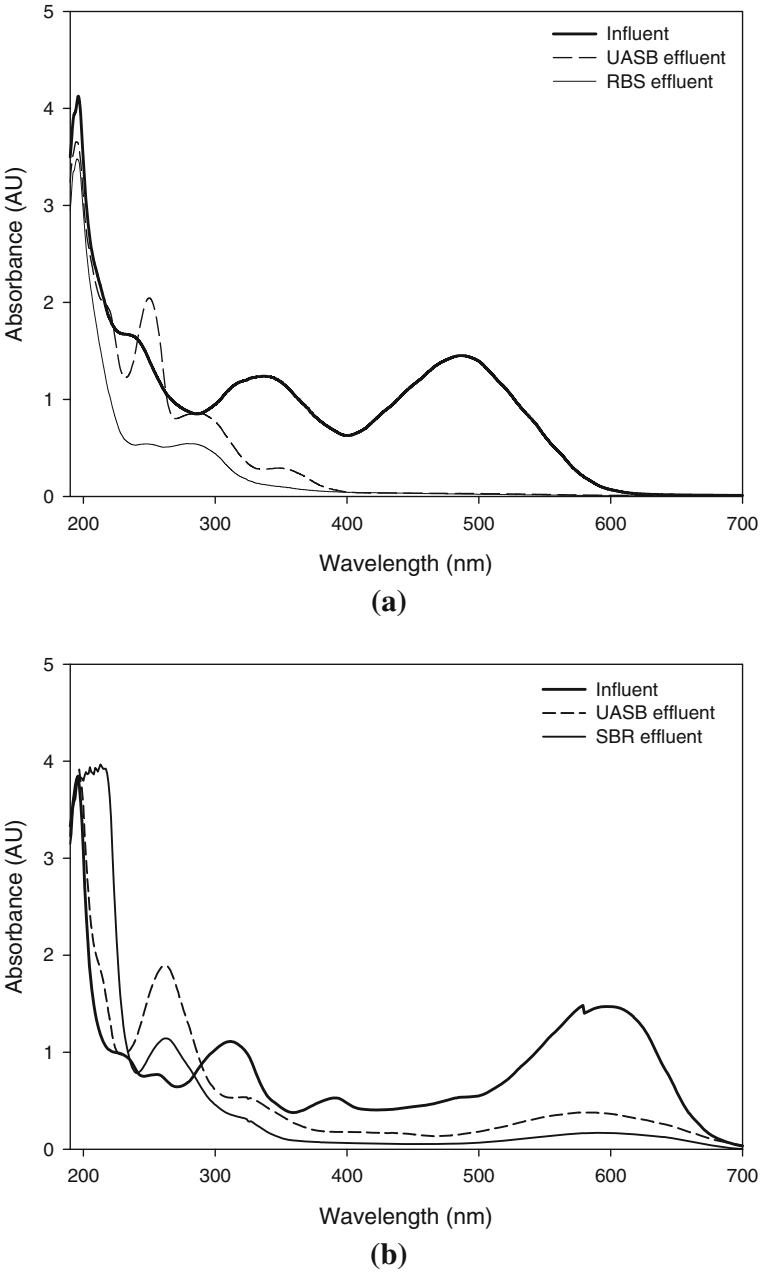


Fig. 2 Absorbance spectra of influent and effluent samples from the anaerobic and aerobic reactors for the synthetic wastewaters containing CR (**a**) and RB5 (**b**)

anaerobic by-products of the reactive dyes RB5 and Reactive Violet 5 [16–18]. Sponza and Işik [21] also noticed that approximately 50% of the aromatic amines from the benzidine-based dye Direct Black 38 could not be removed under aerobic conditions.

COD Removal and Operational Stability

The results of COD influent and effluent concentrations, as well as SBR efficiencies, are shown in Fig. 3.

After an acclimatisation period with a dye-free synthetic wastewater, the SBR was fed with anaerobic CR-containing effluent. A fast decrease in COD removal efficiency was observed when SBR started treating the effluent, and the aerobic reactor collapsed in less than 1 week (data not shown). During this period, SBR pH increased progressively, reaching values close to 8.5. In order to evaluate if the toxicity was permanent or not, a new

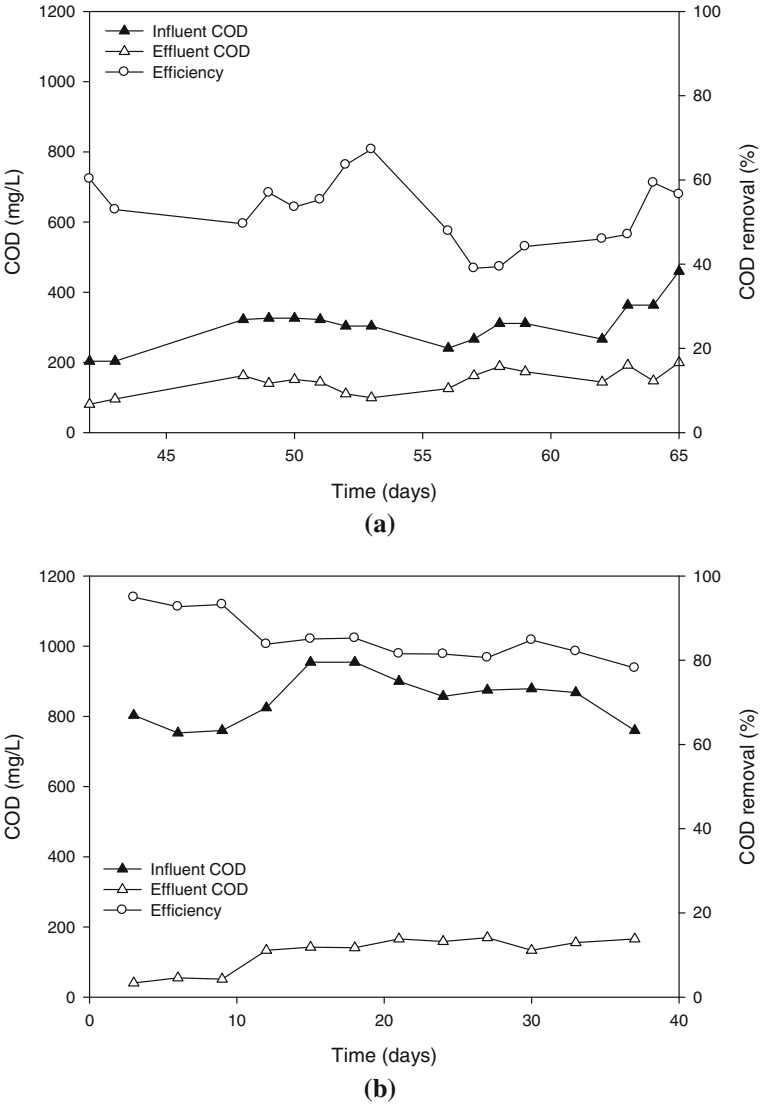


Fig. 3 SBR COD removal performance during the treatment of the synthetic wastewaters containing the dyes CR (**a**) and RB5 (**b**) pre-treated in the UASB reactor

acclimatisation period was started, in which SBR was fed again with a dye-free basal medium. A quick recovery was observed, and a COD removal close to 100% was found in less than 10 days, which indicated that the toxicity was not permanent.

Once more, SBR started receiving CR-containing effluent from the UASB reactor, but in this period, pH was corrected, which resulted in COD removals of 52.4% and 88.1%, respectively, for SBR and anaerobic/aerobic system. In addition, the SBR was shown to be more stable, but some fluctuations in COD removal efficiency were still found (Fig. 3a). Such behaviour evidenced that inhibition was not caused by pH increase but probably by either aromatic amines generated during anaerobic reduction of CR, such as benzidine (Fig. 1), which is reported to be very toxic, carcinogenic and mutagenic, being able to damage microbial activity [41], or by-products formed during aromatic amines autoxidation, which may also be toxic and mutagenic [12].

However, Sponza and Işik [22] did not observe any inhibitory effects of dye (Direct Red 28) intermediates (aromatic amines, especially benzidine) on the aerobic microbiota, which resulted in COD removal efficiencies of 63% and 91% respectively for the aerobic reactor and the sequential anaerobic/aerobic system. Kapdan et al. [42] also did not report inhibition in their activated sludge system, which treated an anaerobic synthetic wastewater containing 100 mg/L of the azo dye Reactive Red 195, unless when high COD concentrations (>7,000 mg/L) were applied, which might have caused a shock effect on microorganisms in the activated sludge unit and caused substrate inhibition resulting in 20% COD removal efficiency.

For the azo dye RB5, the aerobic step presented a high average COD removal efficiency during this experiment (85.3%), reaching values higher than 90% during the initial 10 days (Fig. 3b). These results show the aerobic step importance to reduce COD in anaerobic/aerobic treatments of azo dye-containing wastewaters as, for RB5, average COD removal increased from 28.1% (aerobic phase influent) to 88.0% (after the anaerobic/aerobic system).

Similar results were obtained by Kapdan and Oztekin [43] that studied the application of an anaerobic (HRT of 4 h)/aerobic (HRT of 20 h) system to treat a synthetic Remazol Rot-containing wastewater (70 mg/L) with glucose as carbon source. They found COD removal of 50% during anaerobic phase and reached 80% after aerobic treatment.

Sponza and Işik [20], using a UASB reactor followed by an activated sludge system to treat a synthetic wastewater containing the azo dye RB5 (dye load between 10 and 35 $g/m^3 \cdot day$), verified reductions up to 96% of COD influent, and 85–95% of UASB COD effluent were removed during aerobic treatment.

The statistical tests revealed that there was a significant difference ($p<0.001$) between the COD removal efficiencies achieved by the SBR during the experiments with both dyes, i.e. the reactor was more efficient for the dye RB5. However, regarding the overall COD removal efficiency, the system had a similar performance ($p=1.000$) for both experimental periods (~88%). Furthermore, the final effluent COD concentrations were not statistically different ($p=0.416$) even though the average values were 145 and 126 mg/L for CR and RB5, respectively.

Therefore, it can be concluded that the anaerobic/aerobic sequential treatment used in the present investigation was efficient at COD removal.

Azo Dye Detoxification Using Anaerobic/Aerobic Wastewater Treatment Systems

Initial acute toxicity tests with *D. magna* were carried out with CR. No toxicity was found with CR influent, i.e. the different dilutions used did not cause any death or immobility of *D. magna*, and, therefore, the EC_{50} could not be calculated (Table 2). However, for the UASB

Table 2 EC_{50} values obtained with the acute ecotoxicity tests with *D. magna*

Wastewater	Sample	EC_{50} (%)
CR-containing wastewater	Influent	N.T.
	Anaerobic effluent	2.12
	Aerobic effluent	6.87
RB5-containing wastewater	Influent	23.02
	Anaerobic effluent	40.34
	Aerobic effluent	N.T.

NT not toxic

effluent, a very low EC_{50} was found (2.12%) (Table 2), which evidenced a high toxicity of the CR by-products, i.e. aromatic amines such as benzidine (Fig. 1) [22, 41]. The aerobic post-treatment was not able to considerably decrease the toxicity problem, in which EC_{50} only increased to 6.87%, suggesting that either only a small fraction of the aromatic amines was mineralised under aerobic conditions [44] or the autoxidation by-products were more recalcitrant to biological degradation and still presented a high toxicity [5, 12]. This reinforces the aerobic microbiota inhibition hypothesis by the CR by-products as discussed in section "COD Removal and Operational Stability".

These results partially agree with Sponza and Işik [22], who evaluated the acute toxicity with *D. magna* of synthetic effluents containing the azo dye DR28 after anaerobic/aerobic biological treatments. Their results showed that azo dye by-products generated during the anaerobic treatment were very toxic to *D. magna*, and toxicity was removed after aerobic post-treatment, indicating that, probably, compounds which were causing the toxicity were partially degraded under aerobic conditions.

However, the above-mentioned authors still reported that toxicity values of aerobic effluents were significantly lower than anaerobic effluents, resulting in a significant mineralization of the carcinogenic benzidine even when this amine was at high concentrations in aerobic stage, which did not occur in the present investigation.

The same toxicity tests were conducted with RB5. In contrast to the experiments with CR, RB5 influent caused toxicity in *D. magna*, and the EC_{50} was 23.02% (Table 2). The anaerobic step was capable of decreasing toxicity (EC_{50}=40.34%), which also contradicts the findings with CR (Table 2). The findings with RB5 agree with the results presented in section "COD Removal and Operational Stability", which indicates that RB5 might be more toxic than CR for the anaerobic microorganisms. However, the aerobic step was very effective in removing any trace of RB5 toxicity in *D. magna*, showing that indeed the aerobic phase was capable of reducing COD and toxicity.

The results are in agreement with the observations of Franciscon et al. [28, 29], who reported that the toxicity of RB5 in *D. magna* decreased with the microaerophilic treatment and was completely removed after the aerobic stage as a significant amine concentration reduction occurred.

Conclusions

Colour removal of CR and RB5 mainly occurred during anaerobic phase, achieving average efficiencies higher than 90% and 75%, respectively.

Anaerobic/aerobic biological treatment considerably removed COD of the synthetic wastewaters containing azo dyes such as CR and RB5. However, in one hand, the toxicity of RB5 by-products affected COD removal during anaerobic step as VFA accumulation was

observed; on the other hand, CR by-products affected COD removal during aerobic step as COD efficiency was lower.

Ecotoxicity tests with *D. magna* as the indicator-organism with the azo dye CR revealed that anaerobic effluent presented a higher toxicity compared with the influent, and an aerobic post-treatment was not efficient in reducing this toxicity. However, the results with the azo dye RB5 showed that both anaerobic and aerobic steps could decrease dye toxicity, especially the aerobic phase, which completely removed the toxicity in *D. magna*. Therefore, the anaerobic/aerobic treatment is not always effective in detoxifying dye-containing wastewaters, sometimes even increasing dye toxicity.

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