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Colour removal of dyes from synthetic and real textile wastewaters in one- and two-stage anaerobic systems

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ABSTRACT

Decolourisation of the azo dye model compound, Congo Red (CR), and real textile wastewater, was assessed in one- and two-stage anaerobic treatment systems (R_1 and R_2, respectively). High colour removals were achieved in both treatment systems even when a very high CR concentration (1.2 mM) was applied. However, R_2 presented a slightly better stability, in which the acidogenic reactor ($R_{2,A}$) played a major role on dye reduction, as compared to the methanogenic reactor ($R_{2,M}$), evidencing the role of fermentative microorganisms. The minimum electron donor concentration required to sustain dye reduction was much higher than the stoichiometric amount. Additionally, a decrease on the hydraulic retention time (from 24 to 12 h) did not significantly affect decolourisation, indicating that electron transfer was not a concern. Finally, experiments with real textile wastewater showed low decolourisation efficiencies in both systems, most likely due to the presence of dyes not susceptible to reductive decolourisation under these experimental conditions.

1. Introduction

The textile industry represents an important economic sector worldwide, being responsible for 1.7% of world exportation in 2007, which corresponded to the amount of US$ 238.1 billions (WTO, 2008). Thus, with the increased demand for textile products in last decades, a proportional wastewater generation increase was observed, through which large amounts of dyes and other chemicals are released into superficial waters (dos Santos et al., 2007).

In particular, coloured effluents release into the environment is undesirable not only for affecting the aesthetics, the water transparency and the gas solubility in water bodies, but also because many dyes and their breakdown products are toxic, mutagenic or carcinogenic (Banat et al., 1996; Weisburger, 2002).

Colour removal of these compounds is still one of the most difficult tasks faced by wastewaters treatment plants (WTP) of textile factories, mainly because dyes and pigments are designed to resist biodegradation, 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).

Amongst the different decolourisation methods, the biological treatment has called attention for being economically attractive. However, colour removal by aerobic bacteria, such as those commonly present in activated sludge systems, is normally low (dos Santos et al., 2007), which is mainly associated with the dye adsorption in the sludge (Alinsafi et al., 2006). On the other hand, under anaerobic conditions, effective dye decolourisation can be reached (Van der Zee et al., 2001).

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

Because dye reduction competes with methanogenesis for the same electrons generated upon electron donor oxidation (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, since the probability of the electrons to be channeled to dye reduction would be higher. However, two-stage anaerobic treatment systems have scarcely been applied for textile wastewater decolourisation.

The main goal of the present investigation was to assess the decolourisation of the azo dye Congo Red (CR) and real textile wastewater in one- and two-stage anaerobic systems under different operational conditions, such as different CR and electron donor (ethanol) concentrations, and different hydraulic retention times (HRT).

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2. Methods

2.1. Reactors

The UASB reactors were made of PVC tubes and connections for sewage. The one-stage system (R_1) (Fig. 1) consisted of only one reactor while the two-stage system (R_2) (Fig. 2) consisted of an acidogenic reactor ($R_{2,A}$), a settler and a methanogenic reactor ($R_{2,M}$).

The more relevant reactors dimensions are shown in Table 1. With the exception of $R_{2,A}$, the other reactors had a modified gas–solid-liquid separator (Y-shaped). 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.

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), after which methane was measured in a Mariotte flask (liquid displacement method).

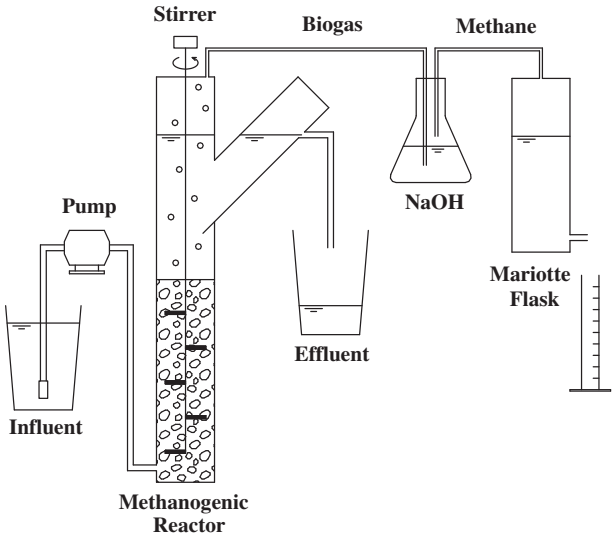

Fig. 1. Schematic of the one-stage anaerobic system (R_1).

2.2. Seed inoculum

The anaerobic sludge used in this experiment was collected from a brewery mesophilic UASB reactor (Insustrial District, Ceará, Brazil). The sludge volume added in the reactors provided a sludge concentration of approximately 30 g VSS/L.

2.3. Synthetic textile wastewater

The synthetic wastewater was composed of distilled water, dye, carbon source (electron donor), basal medium (nutrients) and a buffer. The dye used was Congo Red (CR) (or Direct Red 28, DR28) (analytical grade, Vetec, Brazil), a direct diazo benzidine-containing dye. Ethanol was selected as the electron donor (99.8% purity, Dinâmica, Brazil). The basal medium consisted of (mg/L): NH_4Cl (280), K_2HPO_4 (250), $MgSO_4{\cdot}7H_2O$ (100) and $CaCl_2{\cdot}2H_2O$ (10) and 1 mL/L of trace elements containing (mg/L): 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 HCl 36% (1). 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.

2.4. Real textile wastewater

Textile wastewater was collected once a week from a cotton-processing factory (Industrial District, Ceará, Brazil). It is worth mentioning that the dyeing process of this factory is continuous and utilizes many different reactive dyes. Because the pH value was usually alkaline (~10) the pH was adjusted to 7.0 with sulphuric acid (H_2SO_4), after which electron donor (1.0 g COD/L), nutrients and buffer were added.

2.5. Experimental procedure

The experiment with synthetic textile wastewater was divided in seven periods (Table 2), including reactors start-up (acclimatization period) (period I).

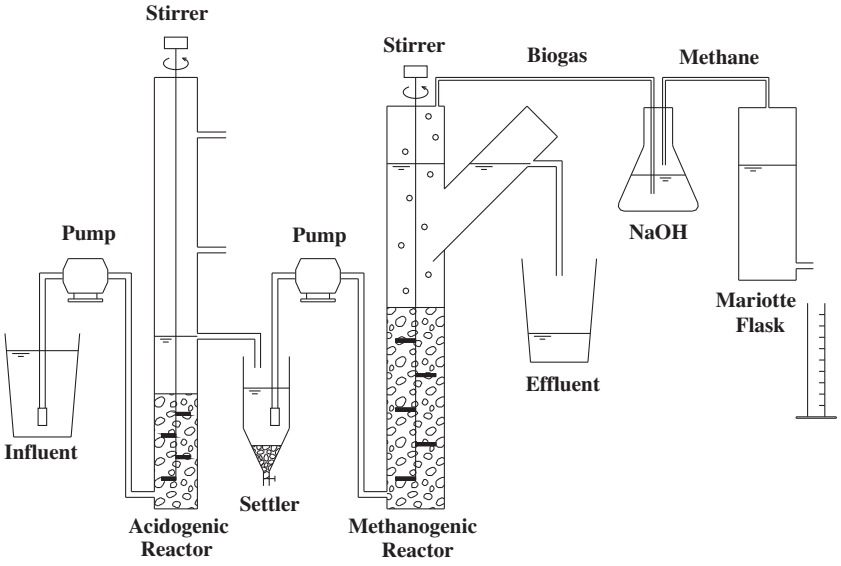

Fig. 2. Schematic of the two-stage anaerobic system R_2 ($R_{2,A}$ and $R_{2,M}$).

Table 1
Reactors dimensions.

Reactor	ϕ (mm)	H_T (cm)	V (L)
R_1	100	70	5.2
$R_{2,A}$	75	75	1.1
$R_{2,M}$	100	70	5.1

ϕ, internal diameter; H_T, total height; V, working volume.

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

	Operational parameters						
Period	I	II	III	IV	V	VI	VII
End of period (days)	30	39	53	70	85	122	143
HRT (h) R_1	24	24	24	24	24	24	12
HRT (h) $R_{2,A}$	4	4	4	4	4	4	2
HRT (h) $R_{2,M}$	20	20	20	20	20	20	10
Substrate (g COD/L)	1.0	1.0	1.0	1.0	0.2	0.5	0.5
CR (mM)	–	0.3	0.6	1.2	1.2	1.2	1.2

After reaching steady operational conditions during the start-up period, CR was introduced at a low concentration of 0.3 mM (~210 mg/L) (period II) in order to avoid a possible microbial inhibition. After verifying reactors stability, CR concentration was increased to 0.6 and 1.2 mM, during the periods III and IV, respectively. In periods V and VI, ethanol concentration was reduced to 0.2 and 0.5 g COD/L, respectively, to assess the electron donor concentration effect on dye decolourisation. The HRT decrease effect on colour removal was assessed in period VII.

After the latter period was finished, the HRT was re-established and a new acclimatization period took place in a CR-free medium. After this period, the reactors were fed with the real textile wastewater.

2.6. Analyses

Colour was usually analysed three times a week and determined photometrically (Thermo-Nicolet Evolution 100). For the synthetic wastewater, the absorbance was read at wavelength (λ) of 496 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).

For the real wastewater, after sample centrifugation, absorbance was also read at wavelength of maximum absorbance. However, since the wastewater composition was variable, the λ value was not fixed. Hence, a UV–vis scanning was made for each sample to determine the λ of maximum absorbance, which usually ranged between 510 and 580 nm.

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 alkalinity by titrimetric method, all of them according to Standard Methods (APHA, 2005). VFA were determined using Kapp titrimetric method (Buchauer, 1998).

3. Results and discussion

3.1. Synthetic textile wastewater

3.1.1. Colour removal

3.1.1.1. Effect of dye concentration. After the acclimatization period (period I), the dye model compound CR was introduced at a concentration of 0.3 mM (~210 mg/L) (period II) in both systems. The results revealed that their performance, during this period, was very stable (Fig. 3) and no remarkable difference was found between the two treatment systems. Colour removal efficiencies were very high for both systems (Table 3), with values from 95% up to 99%.

These colour removal efficiencies are better than those reported by Rajaguru et al. (2000), who found 95% CR (200 mg/L) decolourisation using an anaerobic filter operated with a HRT of 36 h and 5 g/L of glucose as electron donor (5.35 g COD/L). Therefore, it is verified that, even with the higher HRT and electron donor concentration in the latter work, the colour removal efficiencies reached by R_1 and R_2 in the present study were higher, probably because

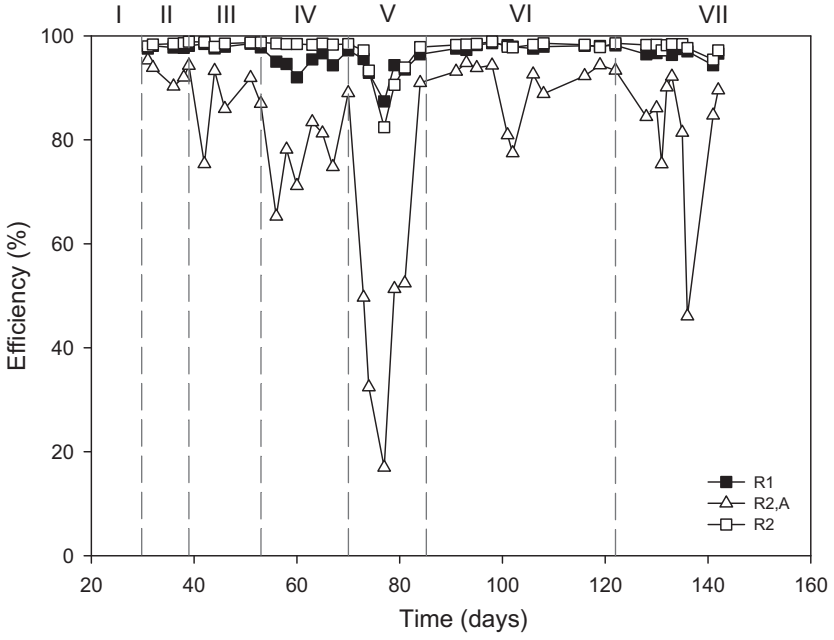

Fig. 3. Colour removal efficiency over the seven experimental periods.

Table 3
Operational performance of the reactors over the experimental periods.

	Operational performance						
Period	I	II	III	IV	V	VI	VII
Colour removal (%) R_1	–	97.8 (0.2)	98.1 (0.4)	95.1 (1.7)	93.4 (3.2)	98.0 (0.4)	96.6 (0.9)
Colour removal (%) $R_{2,A}$	–	93.2 (2.0)	86.7 (7.1)	77.6 (8.0)	49.0 (24.8)	90.6 (5.9)	81.1 (14.1)
Colour removal (%) R_2	–	98.5 (0.3)	98.5 (0.4)	98.4 (0.1)	92.6 (5.6)	98.3 (0.3)	97.7 (1.0)
Influent COD (mg/L) R_1	1400 (90)	1947 (49)	2019 (135)	2148 (168)	1244 (127)	1387 (304)	1154 (135)
Effluent COD (mg/L) R_1	544 (43)	647 (146)	801 (187)	1070 (115)	1067 (191)	1057 (260)	560 (130)
COD removal (%) R_1	61.1 (3.4)	66.7 (7.8)	60.4 (8.8)	49.9 (6.9)	14.7 (8.9)	23.8 (9.2)	50.7 (14.5)
Influent COD (mg/L) R_2	1509 (91)	2026 (75)	2113 (69)	2280 (284)	1313 (191)	1448 (324)	1214 (163)
Effluent COD (mg/L) R_2	614 (209)	467 (107)	645 (179)	869 (77)	946 (239)	979 (292)	561 (111)
COD removal (%) R_2	59.4 (13.6)	77.0 (4.7)	69.3 (9.2)	61.2 (7.3)	27.5 (18.0)	32.5 (11.8)	53.0 (12.3)
pH R_1	7.4 (0.1)	7.6 (0.3)	7.4 (0.3)	7.6 (0.2)	7.4 (0.3)	7.1 (0.2)	7.3 (0.1)
pH $R_{2,A}$	7.3 (0.1)	7.5 (0.4)	7.3 (0.3)	7.6 (0.2)	7.9 (0.4)	7.2 (0.2)	7.4 (0.2)
pH $R_{2,M}$	7.5 (0.1)	7.7 (0.2)	7.4 (0.3)	7.8 (0.2)	7.6 (0.3)	7.1 (0.3)	7.3 (0.1)
TA (mg/L) R_1	629.2 (45.9)	645.0 (35.4)	685.0 (58.7)	709.0 (15.2)	278.3 (12.6)	417.9 (60.3)	503.3 (16.1)
TA (mg/L) $R_{2,A}$	612.5 (49.4)	600.0 (28.3)	645.0 (40.2)	697.0 (67.5)	270.0 (5.0)	438.6 (73.0)	485.0 (26.5)
TA (mg/L) $R_{2,M}$	650.0 (65.2)	640.0 (28.3)	691.3 (74.3)	725.0 (21.8)	285.0 (37.7)	410.7 (48.1)	481.7 (12.6)
VFA (mg/L) R_1	147.5 (53.0)	135.5 (16.4)	255.6 (115.2)	160.8 (83.6)	68.3 (8.3)	204.1 (72.3)	133.2 (30.0)
VFA (mg/L) $R_{2,A}$	154.0 (67.5)	125.1 (1.7)	241.6 (126.9)	206.2 (38.1)	51.3 (12.8)	211.6 (109.6)	186.9 (39.5)
VFA (mg/L) $R_{2,M}$	151.7 (19.0)	188.3 (16.8)	248.6 (108.7)	175.6 (36.5)	59.1 (25.1)	187.1 (64.0)	125.8 (19.3)
VFA/TA R_1	0.23 (0.08)	0.21 (0.01)	0.38 (0.19)	0.23 (0.12)	0.25 (0.04)	0.48 (0.12)	0.26 (0.06)
VFA/TA $R_{2,A}$	0.25 (0.11)	0.21 (0.01)	0.37 (0.20)	0.30 (0.08)	0.19 (0.04)	0.47 (0.17)	0.39 (0.10)
VFA/TA $R_{2,M}$	0.24 (0.04)	0.29 (0.01)	0.37 (0.19)	0.24 (0.06)	0.20 (0.06)	0.45 (0.12)	0.26 (0.03)

The standard deviation is shown in parenthesis.

of the higher biomass concentration contained in the UASB reactors compared to fixed-film systems (Van der Zee and Villaverde, 2005).

Despite showing minor instability (Fig. 3), the acidogenic reactor ($R_{2,A}$) achieved colour removal efficiencies higher than 90% (Table 3), even when operated with a HRT of only 4 h. Therefore, the acidogenic reactor was the main responsible for the total decolourisation obtained by the two-stage system. The hypothesis of utilizing a two-stage reactor is based on the fact that methanogenesis competes with CR for the same electrons generated upon substrate oxidation. Thus, by separating the two processes, the probability of the electrons to be channelled to the dye would be higher, which would enhance colour removal. However, in the present experiment, CR was easily reduced, which probably masked the effect of separation of acidogenic and methanogenic phases.

Talarposhti et al. (2001) used a two-stage system in the treatment of a synthetic wastewater composed by a mixture of seven basic dyes (1000 mg/L) with whey powder as the electron donor. The system consisted of a stirred acidogenic tank (HRT = 24 h) followed by an anaerobic filter (HRT = 48 h). They also verified the important contribution of acidogenesis to the colour removal process, which was responsible for approximately 54% of the total colour removal efficiency (74%) of the two-stage system. Therefore, the findings of the present investigation are in accordance with the above mentioned work.

In period III, CR concentration was increased to 0.6 mM (~420 mg/L) but the system performance was similar to that observed in the previous period, even though $R_{2,A}$ was less stable and its average colour removal efficiency dropped from 93.2 to 86.7%.

When the CR concentration was increased to 1.2 mM (~840 mg/L) (period IV), R_1 showed instability and the decolourisation efficiency dropped from 98.1 (period III) to 95.1%, while R_2 kept the same performance observed in the previous periods. $R_{2,A}$; however, it presented a decrease of nearly 10% in its colour removal efficiency and showed a slightly higher instability than in period III, likely because of the low HRT in the acidogenic phase.

Some studies with UASB reactors also indicated colour removal decrease after the dye concentration was increased (Brás et al., 2005; Sponza and Işik, 2005). Sponza and Işik (2005), for instance, found a decolourisation decrease from 100 to 80% when the concentration of the dye Direct Black 38 was increased from 100 to 3200 mg/L, in a system operated at a HRT between 15 and 16.5 h with glucose as the electron donor (3 g COD/L). Brás et al. (2005) also found a colour removal decrease from 92% to 85% when the Acid Orange 7 concentration increased from 60 mg/L to 300 mg/L in a UASB reactor operated at a HRT of 24 h and supplemented with 1.8 g COD/L of acetate.

Concerning $R_{2,A}$ performance in these two last periods, it is worth mentioning that, in spite of a sudden initial drop on colour removal efficiency after the dye concentration was increased, a fast recovery was found, which indicates that this inoculum adapted to the new operational conditions.

3.1.1.2. Effect of electrons donor concentration. Theoretically, two pairs of electrons are required to reduce an azo bond (–N=N–), i.e. 32 mg COD per mmol of monoazo dye (Van der Zee and Villaverde, 2005). Thus, to reduce 1.2 mM of CR (diazo), it is necessary 76.8 mg COD/L, which represents less than 10% of the supplemented COD as electron donor (1 g/L), during the first four periods of this experiment.

Ethanol concentration was reduced to 0.2 g COD/L (period V), which was still higher than the stoichiometric concentration required for CR reduction. Data shown in Fig. 3 indicates a continuous colour removal decrease in the reactors, especially in $R_{2,A}$, during the first seven days, and a subsequent gradual increase, reaching values close or slightly higher than those obtained at the end of the previous period.

Initially, these results suggest that the efficiency drop can be due to the smaller concentration of available substrate, which affected dye reduction kinetics (Van der Zee and Villaverde, 2005). However, it is also possible that an electron donor competition between dye-reducing and non-dye-reducing microorganisms has occurred, and, therefore, fewer electrons were channelled to reduce the dye (dos Santos et al., 2006).

Later, in period VI, ethanol concentration was increased to 0.5 g COD/L, which positively affected the reactors performance in terms of colour removal efficiency (Table 3) and stability, noticeably $R_{2,A}$ (Fig. 3). R_1 performance in this period was close to that obtained in R_2.

In the present investigation, a direct effect between the electrons donor concentration and the decolourisation efficiency was observed, since the anaerobic consortium tested was incapable of using CR as a carbon and energy source, therefore an electron donor dosage was needed. However, literature reports contradictory results on the effect of substrate concentration. For instance, Ong et al. (2005b) used an anaerobic sequencing batch reactor (SBR) (reaction time = 21.5 h) and found an increase from 30 to 80% on Acid Orange 7 (50 mg/L) decolourisation by doubling the concentration of a mixture of bacto-peptone and sucrose (1:3). Işik and Sponza (2005a) used a UASB reactor (HRT = 18–19 h) and did not observe any change in the CR (100 mg/L) decolourisation when glucose concentration increased from 100 to 500 mg COD/L. However, it is important mentioning that studies with low concentrations of easily reduced dyes associated to a high substrate concentration do not tend to reveal major differences on colour removal.

3.1.1.3. Effect of HRT. By decreasing the HRT of both reactors by half (period VII), with the exception of $R_{2,A}$, both systems were more unstable than in the previous period, i.e. a higher standard deviation, even though the efficiency averages were very close (Table 3). Once again reactor R_2 was more efficient than R_1, and the acidogenic stage ($R_{2,A}$) was responsible for about 83% of the total decolourisation of the two-stage system, even when it was operated with a HRT of only 2 h.

The results revealed that CR was not a very recalcitrant dye, since a colour removal higher than 95% could be found with a short HRT (about 8 h) (Costa et al., 2010). dos Santos et al. (2005b) evaluated the decolourisation of Reactive Red 2 (1250 mg/L) in a EGSB reactor, and found that the HRT decrease from 10 to 5 and, then, to 2.5 h, decreased the colour removal efficiencies from 56 to 37 and to 13%, respectively, therefore suggesting that the HRTs used were not long enough to allow dye reduction satisfactorily.

Since the effect of HRT on decolourisation may be influenced by the dye structure and the type of reactor, different results are reported in literature (Sandhya et al., 2005; Sponza and Işik, 2002; Talarposhti et al., 2001). For instance, Sponza and Işik (2002) studied the azo dye Reactive Black 5 (100 mg/L) decolourisation in a UASB reactor using 3 g COD/L of glucose as electron donor, and found a decrease from 97 to 87% when the HRT was reduced from 30 to 3 h. In contrast, Sandhya et al. (2005) observed a reduction of only 1% (from 84 to 83%) when the HRT of an anaerobic filter decreased from 10 to only 3.6 h, while treating the azo dye Golden Yellow (50 mg/L) with glucose (100 mg/L) and yeast extract (200 mg/L) as electron donors.

3.1.2. COD removal and operational stability

Operational data for reactors performance are shown in Table 3, 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.

COD removal efficiency of R_1 decreased with CR concentration increase (periods II, III and IV), which could indicate microbial inhibition by dye toxicity. However, a gradual increase can also be observed in influent and effluent COD values, which corresponded to the additional amount of dye in each period.

According to Işik and Sponza (2005a), 100 mg/L of CR are equivalent to a COD of 74.6 mg/L. Therefore, 210 and 420 mg/L (0.3 and 0.6 mM) of CR are equivalent to, respectively, 156.7 and 313.3 mg COD/L, which corresponds approximately to the arithmetic difference between the average values of the effluent COD in periods II and III (153.9 mg/L) and between the values in periods III and IV (269.4 mg/L) of R_1. Thus, the difference between the initial and final COD value kept practically constant. The same behaviour can be observed in R_2.

Brás et al. (2005) also found COD efficiency decreased from 92 to 67% when the Acid Orange 7 concentration increased from 60 mg/L to 300 mg/L in a UASB reactor. However, the electron donor conversion was not affected since no acetate accumulation was found. Hence, the authors concluded that the residual COD could be attributed to non-reduced dye or its metabolites (aromatic amines).

During periods IV, V and VI, when ethanol concentration varied, a considerable drop in the COD removal efficiency was found. It is clearly noticed that the effluent concentration was practically unaltered, i.e. the recalcitrant fraction, in which CR is included, remained the same because there was no change in its composition. Thus, the average efficiency simply changed because the amount of influent biodegradable organic matter (ethanol) varied. These results are in agreement with the ones found in Işik and Sponza (2005a), who used a UASB reactor treating a CR-containing synthetic wastewater (100 mg/L) and observed a COD removal efficiency decrease from 78 to 68% by decreasing the glucose concentration (electrons donor) from 500 to 100 mg/L. On the other hand, Kapdan and Oztekin (2006) studied an anaerobic SBR to remove the dye Remazol Red RR (60 mg/L) and found a COD removal efficiency decrease from about 70 to 45% when the initial COD (glucose) was between 400 and 800 mg/L, and above 1300 mg/L, respectively.

Later, when the reactors HRT was reduced by half (period VII), a total COD removal efficiency increase was observed in relation to the previous period, although the influent composition was the same. The final COD was also smaller than the one achieved in previous periods. From these results, it can be suggested that decreasing HRT the contact time between sludge and aromatic amines formed upon CR reduction would be shorter. So, the toxic effect of the breakdown products might have decreased, which increased COD removal.

Several studies report a positive relation between HRT and COD removal (Brás et al., 2005; dos Santos et al., 2005b; Işik and Sponza, 2005b, 2008; Kapdan and Oztekin, 2006; Ong et al., 2005a; Sandhya et al., 2005). For example, Işik and Sponza (2005b) observed a COD removal reduction while treating a synthetic textile wastewater containing a mixture of five azo dyes (250 mg/L, 50 mg/L each) in a UASB reactor. The average efficiency dropped from 79.9 to 29.4% when the HRT was decreased from 100 to 6 h. However, Sponza and Işik (2002) found contradictory results when the HRT of a UASB reactor treating the azo dye Reactive Black 5 (100 mg/L) was decreased from 30 (40.8%) to 15.7 h (56%) and, then, to 3 h (26.6%). Çinar et al. (2008), although using a different treatment system (an anaerobic–aerobic SBR) to decolourise 100 mg/L of the azo dye Reactive Violet 5, also observed a similar behaviour. When the anaerobic phase of the reactor decreased from 24 to 12 h, the average COD removal changed from 87 to 78%, but it increased to 84% when the anaerobic reaction time was decreased to 6 h.

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.

3.2. Real textile wastewater

3.2.1. Colour removal

Colour removal efficiencies and absorbance values for influent and effluent are shown in Table 4. It can be noted that the decolourisation efficiency presented considerable variation, which can not be completely attributed to the reactors instability because

Table 4
Average absorbance and colour removal efficiency.

Reactor	Influent absorbance	Effluent absorbance	Efficiency (%)
R_1	0.490 (0.138)	0.198 (0.026)	57.1 (12.3)
$R_{2,A}$	0.493 (0.117)	0.267 (0.044)	44.8 (5.9)
R_2	0.493 (0.117)	0.237 (0.060)	50.1 (16.9)

The standard deviation is shown in parenthesis.

Table 5
Average values of influent and effluent COD and average removal efficiency.

Reactor	Influent COD (mg/L)	Effluent COD (mg/L)	Efficiency (%)
R_1	1532.7 (248.5)	601.7 (131.3)	60.3 (8.1)
R_2	1640.7 (271.3)	581.0 (328.5)	65.5 (15.3)

The standard deviation is shown in parenthesis.

the wastewater was collected weekly and varied with the factory production process; its composition changed and influenced influent absorbance values. However, the effluent absorbance was usually close to 0.2. Therefore, the lower influent absorbance, the lower the registered colour removal efficiency. Although colour removal efficiencies were smaller than the ones found with synthetic wastewater, the systems were efficient with both wastewaters.

Somasiri et al. (2008) used a UASB reactor (HRT = 24 h) supplemented with nutrients and glucose to treat a real textile wastewater (COD_{total} = 6 g/L) and found 95% colour removal, even when the initial absorbance at wavelength of maximum absorbance (λ = 580 nm) was only 0.35.On the other hand, Şen and Demirer (2003) used a fluidized bed reactor (FBR) operated at a HRT of 24 h to treat a real textile wastewater supplemented with glucose (500 mg/L) and nutrients and achieved colour removal efficiencies between 40 and 44%.

It is important to comment that, for some textile wastewaters, absorbance measurement is difficult due to the absence of expressive peaks in their visible spectrum. The low colour removal efficiencies found in the present investigation can be a result of this behaviour. Additionally, the probable presence of a considerable amount of anthraquinonic dyes in the utilized wastewater might have also been responsible for the low decolourisation reached, as these dyes are more recalcitrant under reductive conditions than azo compounds (Carliell et al., 1994; dos Santos et al., 2005a).

Concerning the systems performance, colour removal was slightly higher in the reactor R_1 (7% difference), which could be attributed to the low HRT of 4 h in this phase for the acidogenic reactor ($R_{2,A}$). In other words, the short HRT was not enough to reduce the dyes to a tolerable concentration for the methanogenic reactor ($R_{2,M}$), compromising its performance and, consequently, the overall two-stage system performance. Nevertheless, $R_{2,A}$ was responsible for about 90% of colour removal efficiency of the two-stage system. Hence, no concrete evidence was found which may indicate the real reason why R_2 achieved a lower performance than R_1.

Chinwekitvanich et al. (2000) obtained an average decolourisation efficiency of only 14% in the first stage, which corresponded to 25% of the system total decolourisation (57%), while using an acidification tank (HRT = 12 h) followed by a UASB reactor (HRT = 12 h) to treat a diluted reactive red dyeing wastewater (~1:6.5), supplemented with 500 mg/L of tapioca and nutrients. However, the acidification tank used in their experiment was not previously inoculated with any kind of microorganism, which probably justifies the low colour removal achieved in this unit.

The results of the present investigation suggest that the acidogenic microorganisms are the principal responsible for the colour removal under anaerobic conditions, which are in agreement of the findings of dos Santos et al. (2006).

3.2.2. COD removal and operational stability

The pH varied between 7.0 and 8.0, and the reactors also showed a good operational stability, since their total alkalinity and VFA values were around 900 and 200 mg/L, respectively.

Concerning COD removal (Table 5), the efficiencies were nearly 60% and final effluent COD was similar to the concentration obtained in periods II and III with synthetic wastewater, when CR concentrations were lower. This suggests that the dye concentration in the real textile wastewater, in other words, its recalcitrant COD, were far lower than the CR concentrations tested (periods IV–VII).

Somasiri et al. (2008) used a UASB reactor (HRT = 24 h) supplemented with nutrients and glucose to treat a real textile wastewater (COD_{total} = 6 g/L) and obtained a COD removal above 97%, and effluent COD of 200 mg/L, which was lower than the ones from the present investigation (~500 mg/L).

In contrast, Şen and Demirer (2003) tested a FBR (HRT = 24 h) to decolourise a real textile wastewater supplemented with glucose (500 mg/L) and nutrients and found COD removal efficiencies between 62 and 66%, which are close to the findings of the present work.

4. Conclusions

Excellent colour removals were achieved even for the very high dye concentration tested.

R_2 presented a slightly better stability, in which $R_{2,A}$ was the main responsible for decolourisation, evidencing the role of fermentative microorganisms on dye reduction.

The minimum electron donor concentration required to sustain dye reduction was much higher than the stoichiometric amount.

HRT reduction did not affect dye decolourisation. Thus, electron transfer was not a concern in the present investigation.

The experiment with real textile wastewater showed lower decolourisation efficiencies, most likely due to the probable presence of a considerable amount of anthraquinonic dyes.

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