





Biological treatment of real textile wastewater containing sulphate, salinity, and surfactant through an anaerobic–aerobic system

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ABSTRACT

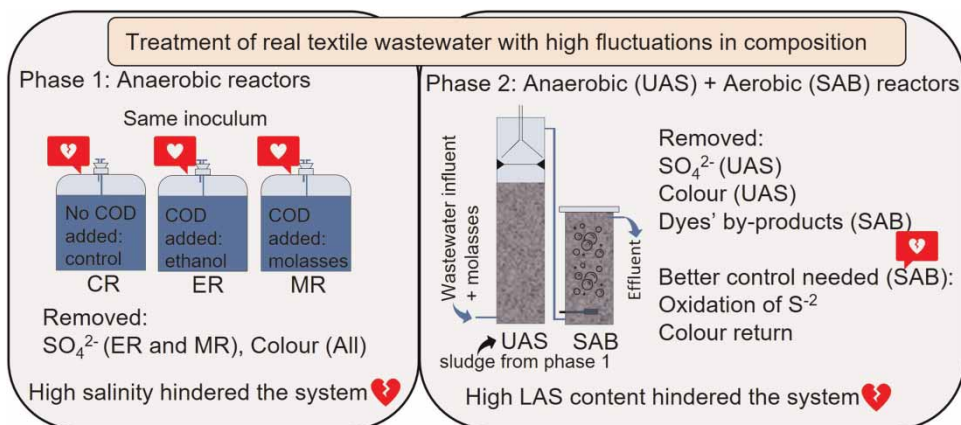
Real textile wastewater containing high salinity (up to 12.6 g·kg⁻¹) and surfactant (up to 5.9 mg·L⁻¹ of linear alkylbenzene sulfonate – LAS) was submitted to biological treatment for colour (up to 652 mg Pt-Co·L⁻¹) and sulphate (up to 1,568.6 mg SO₄²⁻·L⁻¹) removal. The influence of ethanol and molasses supplementation was firstly evaluated in anaerobic batch reactors for the removal of dyes and sulphate. Subsequently, aiming to remove aromatic amines (dye degradation by-products), an anaerobic–aerobic continuous system supplemented with molasses was applied. Supplementation had no influence on colour removal (maximum efficiencies around 70%), while it improved sulphate reduction (23% without supplementation against 87% with supplementation), and conferred robustness to the reactors, which recovered quickly after higher salinity impact. The aerobic reactor removed aromatic amines when the level of surfactants was lower than 1.0 mg LAS·L⁻¹, but the performance of the system was hindered when the concentration was increased to 5.9 mg LAS·L⁻¹. Findings suggest that the supplementation of an easily biodegradable organic matter might be a strategy to overcome wastewater fluctuation in composition.

Key words: anaerobic co-digestion, azo dyes, COD/sulphate ratio, external carbon source, interferences, LAS

HIGHLIGHTS

- The characteristics of real textile wastewater vary with the industrial process type and may contain interferences that harm biological treatment.
- Interferences are related to high levels of salinity and surfactants.
- Good treatment efficiency of colourful wastewater was obtained by applying an anaerobic–aerobic system and electron source supplementation.
- To overcome interferences we propose strategies for industrial practices.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Textile wastewaters are mainly characterized by high colour and salinity contents, and sometimes, high sulphate levels. Wild fluctuations are usually reported for other parameters, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD) and pH (Santos *et al.* 2007).

Colour in wastewater is a result of unfixed dyes released from different industrial processes (Santos *et al.* 2007; Sudha *et al.* 2014; Köchling *et al.* 2016). As pollutants in water bodies, dyes produce undesirable aesthetic problems and can compromise water transparency (Somāsiri *et al.* 2008). Additionally, they are toxic to aquatic life (Chung & Stevens 1993) and some of their degradation by-products are known to be carcinogenic and/or mutagenic (Brown & DeVito 1993).

To achieve the maximum fixation of the dyes to the fibre, high concentrations of salts, such as sodium nitrate, sodium sulphate and sodium chloride, are used during the industrial process (Carliell *et al.* 1998). High salinity content in the wastewater can cause negative effects on microbes, compromising biological treatments (Woolard & Irvine 1995).

Due to the chemical characteristics of azo dyes, a common group of dyes used in the textile industry (Santos *et al.* 2007), anaerobic followed by aerobic technologies have been applied for the biological treatment of this kind of wastewater (van der Zee & Villaverde 2005; Pandey *et al.* 2007; Silva *et al.* 2012; Cirik *et al.* 2013; Menezes *et al.* 2019; Oliveira *et al.* 2020; Carvalho *et al.* 2022). Under anaerobic conditions, reduced azo dyes can generate several types of aromatic amines, which may represent a toxic and recalcitrant potential at this stage of the treatment (Carliell *et al.* 1998; Santos *et al.* 2007; Singh & Arora 2011). Aromatic amines, conversely, are well known to be degraded through aerobic processes (Pineiro *et al.* 2004).

Regarding sulphate content, when in high concentration and under anaerobic conditions, sulphate-reducing bacteria (SRB) may compete with the consortium of methanogenic archaea (MA) for electron donors (Damianovic & Foresti 2007; Callado *et al.* 2015). In the context of textile wastewater, dyes and sulphate are both electron acceptors and may compete for the electrons available from organic matter (Santos *et al.* 2007).

It is known that a COD/sulphate ratio ($\text{COD}/\text{SO}_4^{2-}$) of 0.67 is required to achieve complete organic matter removal through sulphate reduction (Lens *et al.* 1998). At higher ratios, other electron acceptors must be available to achieve complete organic matter removal, for instance dyes. For complete sulphate removal, electron donors and carbon sources must be added to sulphate-rich wastewaters which are deficient in COD (Liamleam & Annachatre 2007).

Amaral *et al.* (2014) reported a prevalence of sulphate over dye reduction for the anaerobic treatment of a real textile wastewater. These authors reported that the supplementation with external electron donors may be a strategy to achieve success in both sulphate and colour removal. Hao *et al.* (2014) stated that different electron donors can provide different benefits and drawbacks to the processes, and the choice of the best electron source would depend on the reaction requirements. However, the evaluation of the behaviour of different electron sources for this competition applied to the anaerobic treatment of a real textile wastewater is still scarce.

Liamleam & Annachhatre (2007) highlighted in their review that ethanol was an attractive electron donor for sulphate reduction and that the complete oxidation of ethanol into CO₂ by SRB alone had already been reported. Together with ethanol, molasses was also chosen as an electron donor for the present study due to its low cost and great availability in Brazil, from sugarcane industry. Lactate, a fermentation by-product of molasses, is known to be a common substrate for SRB (Muyzer & Stams 2008). Two major groups of SRB can reduce sulphate into sulphide by the incomplete oxidation of lactate into acetate and CO₂, and by the complete conversion of acetate into CO₂ or HOC₃⁻ (Chen *et al.* 2008).

Regarding the presence of surfactants, this class of compounds is found in softening chemicals used by the industry, and they are also part of chemicals used to improve the binding of the dyes to fibres (Yaseen & Scholz 2019). Interestingly, as far as we know, surfactants are not reported as interference in studies that apply biological treatments of textile wastewater. However, the presence of surfactants and their by-products used in softening processes can impair biological treatment, causing undesirable effects on aquatic life and microbial ecosystems (Motteran *et al.* 2017).

The aim of the present study was to contribute to a better practical understating of an anaerobic–aerobic system applied to the treatment of a real textile wastewater, containing high concentration of salt, sulphate, surfactants, and colour. Considering that most studies in this field were conducted using synthetic effluents, we intend to broaden the comprehension on how those chemical compounds interfere in the biological treatment of a real harsh wastewater. In our study, we also propose strategies to overcome the fluctuations in wastewater content in order to improve the biological treatment performance.

2. MATERIALS AND METHODS

In this study, two phases of a bench-scale experiment were performed to treat a real textile wastewater with high sulphate and salinity content and a mixture of different dyes. In the first phase, under anaerobic conditions, sequential batch reactors were operated to evaluate the influence of two different external electron donors and carbon sources (ethanol and molasses) on sulphate and colour removal.

During the second phase, the complete treatment was applied: the anaerobic stage, consisting of an up-flow anaerobic structured-bed reactor with molasses as external electron donor and carbon source, followed by the aerated stage (submerged biofilter reactor). In addition, the effect of linear alkylbenzene sulfonate (LAS) was observed.

2.1. Real textile wastewater

Real textile wastewater was the substrate of this study. It was provided by a textile laundry industry, which performed the process of jeans degumming, bleaching, dyeing, and softening using many chemical compounds. The textile wastewater was stored in an equalization tank and treated by a physicochemical process in the industry. The substrate for the reactors used in this study was collected approximately every 15 days from the equalization tank of the industry.

2.2. Reactor and operating procedure

2.2.1. Phase 1 – monitoring of the batch reactors

Three glass batch reactors (CR, ER and MR) with 4 L or dm³ of working volume and 2 dm³ of exchange volume were essayed (Figure 1(a)). Reactor CR was the control reactor, in which no external electron source was added. For the others, a COD of 1,200 mg O₂·L⁻¹ was supplemented with ethanol (ER) or molasses (MR). The cycle time was 24 hours (15 minutes of manual feeding, 23 hours of reaction, 30 minutes of settling, and 15 minutes of manual discharging). The reactors were inoculated with 2 g volatile suspended solids (VSS)·L⁻¹ (mixed liquor content) of anaerobic sludge from a pilot-scale upflow anaerobic sludge blanket (UASB) reactor, which was used to treat real textile wastewater (Amaral *et al.* 2014), resulting in a COD/biomass ratio of 0.6 of g O₂·L⁻¹/g VSS. No pre-treatment was applied to the sludge before using it as inoculum. It was just homogenized prior use. The reactors were kept at 30 °C (303 K), in a shaker (Innova, WEG) at 1.25 Hz of orbital agitation.

2.2.2. Phase 2 – monitoring of the continuous reactors

Two continuous-flow reactors were used as an up-flow anaerobic structured-bed reactor (UAS) followed by a submerged aerated biofilter (SAB) (Figure 1(b)). Both reactors (0.55 and 0.35 m in height) were filled with expanded clay balls (2 cm in diameter) to avoid biomass losses, resulting in a working volume of 3 and 2 L or dm³ for UAS and SAB, respectively.

Real textile wastewater supplemented with 1,000 mg O₂·L⁻¹ of COD using molasses was pumped into the UAS reactor at a flow rate of 2 mL·min⁻¹ (hydraulic retention time – HRT – of 1 day). The UAS reactor was inoculated with a mixture of anaerobic sludge from the three previous batch reactors (CR, ER and MR), resulting in a mixed liquor content of 5.5 g

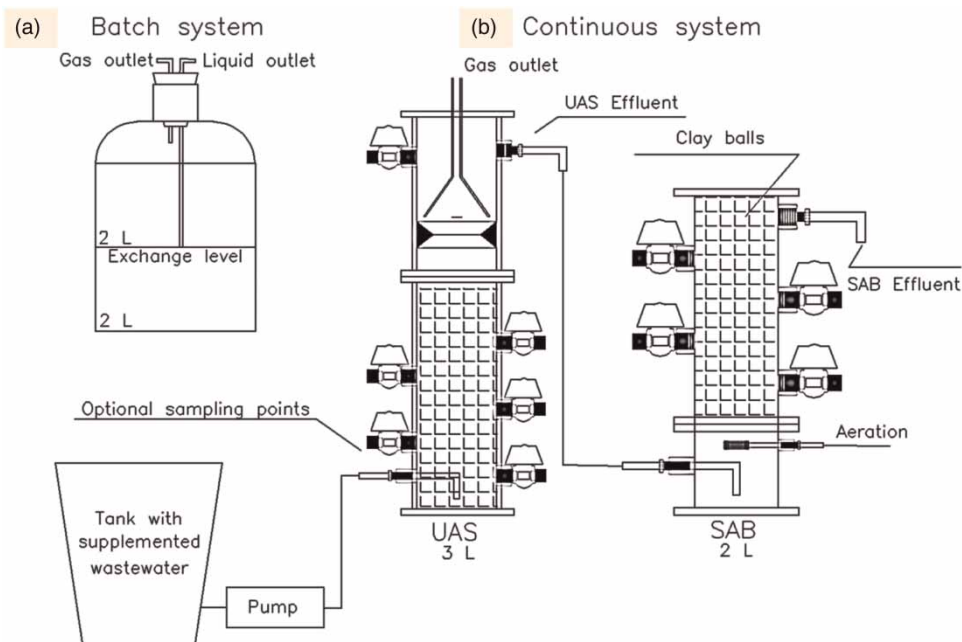


Figure 1 | Diagram of the experimental system and reactors used in phase 1 (a) and in phase 2 (b).

VSS·L⁻¹ and a COD/biomass ratio of around 0.2 g O₂·L⁻¹/g VSS. The aerobic reactor was not inoculated. The dissolved oxygen (DO) in the UAS effluent was below the detection limit during the whole experimental period, while in the effluent from SAB, the DO was increased to 0.16 ± 0.1 mg O₂·L⁻¹ by using an aquarium pump installed at the entry of the aerobic reactor. The anaerobic–aerobic system was maintained at 30 °C (303 K).

2.3. Monitoring parameters

The analytical procedures were conducted following the Standard Methods for the Examination of Water and Wastewater (APHA 2005). Eight lots of wastewater were collected from the industry and characterized in terms of chemical oxygen demand (COD) (SM 5220D), sulphate (SM 4500SO₄²⁻E), alkalinity (SM 2320B), BOD (BOD_{5,20}) (SM 5210BODB), total nitrogen TNK (SM 4500NorgB), ammonia (SM 4500N-NH₃C), phosphorous (SM 4500P D), and colour (SM 2120 C). Salinity and pH were also measured (Hach – HQ40d). For colour determination, the light absorption peak in the VIS spectrum (Hitachi U-2910, Hitachi, Ltd, Tokyo, Japan) was at 667 nm (phase 1) and 573 nm (phase 2); thus, the absorbance at these wavelengths was chosen as an indirect measurement of the sum of different amounts and kinds of dyes in the real wastewater used in phase 1 and phase 2, respectively. Linear alkylbenzene sulfonate was measured as described by Souza *et al.* (2016).

The performance of the reactors was evaluated for 72 days (phase 1) and 75 days (phase 2), by measuring COD, sulphate, sulphide (4500S²⁻F), colour (absorbance), alkalinity, pH, oxidation–reduction potential (ORP) (Hach – HQ40d), and dissolved oxygen (Hach – HQ40d). The values measured for sulphide were discounted from the COD values according to the theoretical relation described by Chan & Farahbakhsh (2015), in which 1,000 mg·L⁻¹ of sulphide (as S) confers 1,996 mg·L⁻¹ of oxygen demand (as O₂). Aromatic amines were qualitatively evaluated during phase 2 by scanning in the range from 200 to 350 nm (Pinheiro *et al.* 2004) twice a week.

At the end of phase 1, the temporal profiles were performed in ER and MR (phase 1), for the COD/SO₄²⁻ ratios of 0.67 and 1.50. The first profile occurred 1 week after the day 72 of phase 1; during the 7 preceding days, both reactors were daily fed with the target COD supplementation and the lot 5 of real wastewater. The same was done for the second profile, which occurred 2 weeks after the end of phase 1. The days before each profile were not monitored. The first analyses were conducted right after feeding the reactors, while the following were conducted every 2 hours, during 24 hours of a typical batch cycle. The COD/SO₄²⁻ ratios were based on the COD added (ethanol or molasses) and the sulphate content in the real wastewater.

For both phases, the efficiencies of reactors were calculated considering the influent wastewater and the effluent of each reactor CR, ER, and MR (phase 1), and UAS and SAB (phase 2). This means that efficiencies removal observed in SAB also represent the global efficiency of the entire system. In this study we present mass concentrations as $\text{mg}\cdot\text{L}^{-1}$ or $\text{g}\cdot\text{L}^{-1}$, which correspond to $10^{-3} \text{ kg}\cdot\text{m}^{-3}$ or $1 \text{ kg}\cdot\text{m}^{-3}$, respectively.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the textile wastewater

Table 1 shows the characterization of the eight lots of wastewater used throughout the experimental period (phases 1 and 2). The textile wastewater content was considerably variable, due to the different processes performed by the industry, in which different chemicals are used. The concentrations ranged from around 500 to 1,600 $\text{mg SO}_4^{2-}\cdot\text{L}^{-1}$, for sulphate; from 4 to 13 $\text{g}\cdot\text{kg}^{-1}$, for salinity; from 250 to 1,000 $\text{mg O}_2\cdot\text{L}^{-1}$, for COD; and from 168 to 652 $\text{mg Pt-Co}\cdot\text{L}^{-1}$ for colour, resulting in 0.083 and 0.463 of minimum and maximum absorbance (both at 667 nm, during phase 1), respectively. The pH ranged from 5.6 to 7.6; when lower than 6.0, it was adjusted to 7.0 using sodium bicarbonate before feeding the reactors. During the second phase, the concentration of LAS was increased from less than 1 to up to 5.9 $\text{mg}\cdot\text{L}^{-1}$. An important fact to consider in the textile wastewater characterized here is the low content of nitrogen and phosphorous. Based on the recommended ratio of 250:5:1 for COD:N:P for anaerobic treatment (Metcalf & Eddy 2003); the eight lots of wastewater used during this study presented sufficient concentrations of nitrogen, but insufficient concentrations of phosphorous in lots 1, 2, 3, 4, 5, and 8. Supplementation of the later macronutrient would be needed to provide more favourable conditions for the microbial community to growth; however, in this study, we assessed only the supplementation of organic matter, to avoid increasing costs to the industry.

Table 1 | Characterization of the eight lots of wastewater used in the study, collected from the industry

	Lots of wastewater collected from the industry							
	1 (phase 1)	2 (phase 1)	3 (phase 1)	4 (phase 1)	5 (phase 1)	6 (phase 2)	7 (phase 2)	8 (phase 2)
COD ($\text{mg O}_2\cdot\text{L}^{-1}$)	497.5 ± 167.5	472.2 ± 167.9	694.1 ± 214.7	991.1 ± 176.8	245.0 ± 61.4	299.4 ± 138.1	416.7 ± 25.3	838.2 ± 54.8
COD/ SO_4^{2-}	0.56 ± 0.2	0.83 ± 0.3	0.63 ± 0.2	0.71 ± 0.1	0.16 ± 0.1	0.34 ± 0.18	0.79 ± 0.05	1.55 ± 0.21
Sulphate ($\text{mg SO}_4^{2-}\cdot\text{L}^{-1}$)	896.1 ± 27.6	573.9 ± 97.4	1,101.0 ± 29.1	1,413.3 ± 374.8	1,568.6 ± 50.8	863.5 ± 84.8	529.4 ± 39.5	547.6 ± 74.7
Colour ($\text{mg Pt-Co}\cdot\text{L}^{-1}$)	168	652	399	462	570	382	345	502
Bicarbonate alkalinity ($\text{mg CaCO}_3\cdot\text{L}^{-1}$)	55.9 ± 2.7	196.8 ± 17.5	0	0	301.8 ± 28.5	237.4 ± 44.3	80.1 ± 22.9	111.9 ± 92.1
Total alkalinity ($\text{mg CaCO}_3\cdot\text{L}^{-1}$)	84.2 ± 7.4	307.6 ± 37.6	109.5	117.3	427.3 ± 75.5	347.0 ± 58.9	145.7 ± 40.6	254.4 ± 172.2
Salinity ($\text{g}\cdot\text{kg}^{-1}$)	7.2 ± 0.2	6.2 ± 0.2	7.5 ± 0.1	12.6 ± 0.2	6.2 ± 0.1	4.7 ± 0.1	3.7 ± 0.1	5.8 ± 0.2
pH	6.8	7.0	5.6	5.7	6.6	7.2	7.0	7.6
BOD _{5,20} ($\text{mg O}_2\cdot\text{L}^{-1}$)	60	140	120	580	160	70	100	310
Nitrogen TKN ($\text{mg N-TKN}\cdot\text{L}^{-1}$)	14.6	14.3	15.5	20.5	21.0	14.7	15.5	22.2
Ammonia ($\text{mg N-NH}_3\cdot\text{L}^{-1}$)	5.8	6.6	5.8	5.3	6.8	3.4	2.7	7.1
Phosphorus ($\text{mg P-PO}_4^{2-}\cdot\text{L}^{-1}$)	0.1	0.8	0.6	0.1	0.3	1.7	3.0	1.9
COD:N:P	250:7.3:0.1	250:7.6:0.4	250:5.6:0.2	250:5.2: < 0.1	250:21.4:0.3	250:12.3:1.4	250:9.3:1.8	250:6.6:0.6
LAS ($\text{mg}\cdot\text{L}^{-1}$)	<1	<1	<1	<1	<1	<1	5.9	2.9

3.2. Phase 1 – monitoring of the batch reactors

Buffer was added during the start-up period of MR, due to the quick fermentation of molasses, by applying the NaHCO_3 :COD ratio of 1:1. Effluent pH of either ER or CR was always higher than 6.0, thus external buffering was not needed (Table 1 of Supplementary Material). In general, the performance behaviour of ER and MR was similar; considering supplementation, influent COD/ SO_4^{2-} ratio varied from 1.0 to 3.7 during the monitoring period, with no lag phase for both COD and sulphate removal.

Considering the entire experimental time, average efficiencies for COD removal were $25 \pm 24\%$, $43 \pm 15\%$, and $43 \pm 16\%$ for CR, ER and MR, while the corresponding values for sulphate removal efficiency were $8 \pm 7\%$, $47 \pm 15\%$, and $44 \pm 14\%$. The highest efficiencies were found after the 25th day of operation, with COD removal of 73 and 68% in ER and MR, respectively (Figure 2(a)), and corresponding values of sulphate removal of 74 and 70% (Figure 2(b)). In CR, an 18-day lag phase period was found for COD removal, achieving the maximum removal efficiency of 79% on the 42nd day of operation (Figure 2(a)). For sulphate, no significant removal was found throughout the operational period in CR, being barely removed from the 29th day (Figure 2(b)).

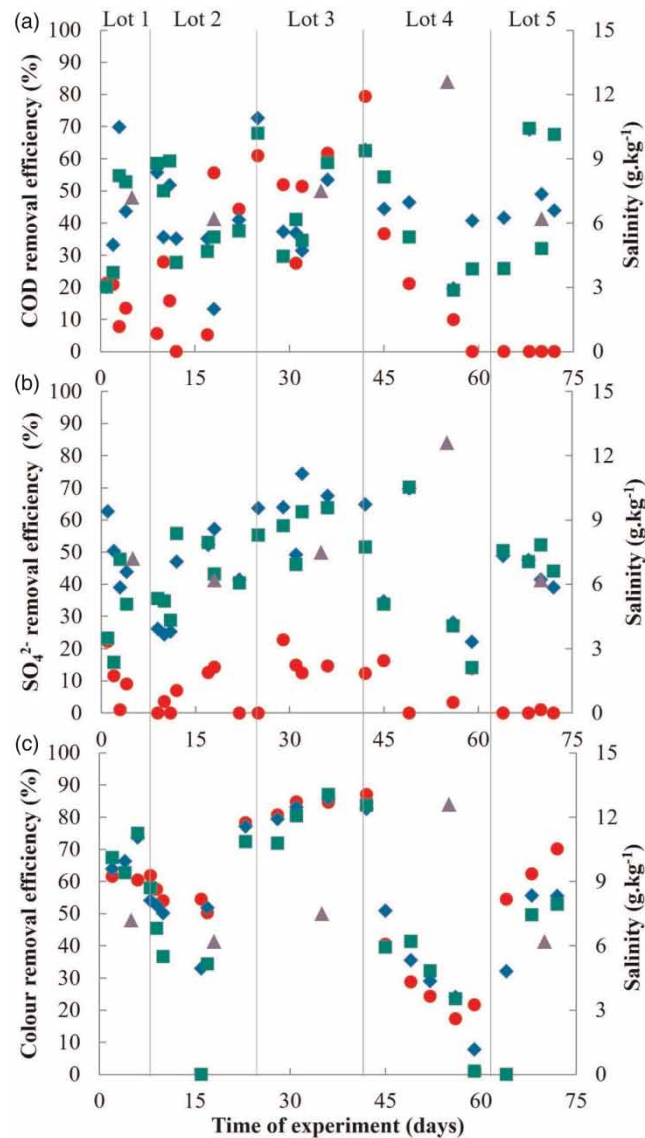


Figure 2 | Efficiencies of COD (a), sulphate (b) and colour (c) removal in CR (●), ER (◆) and MR (■) effluents, and the salinity (▲) of each lot of wastewater used in phase 1.

As it was not possible to measure and analyze the outlet gas from the reactors, some calculations contributed to better understand the COD removed by sulphidogenesis and methanogenesis. The amounts of COD used for sulphidogenesis could be theoretically inferred by using the COD/sulphate ratio of 0.67 for complete organic matter removal through sulphate reduction. The COD amounts used for colour removal could be calculated as well, by using the relation between a tetra-azo dye Direct Black 22 (DB22) and COD proposed by Amorim *et al.* (2013), explained later. Methanogenesis was considered to be responsible for the remaining amounts of COD removed.

It is interesting to note that, during the period of best performance of ER and MR, when lot 3 was used to feed the reactors, the COD removed was almost equally directed to sulphidogenesis and to methanogenesis (around 50% for each). Out of the average COD removal efficiency of 49% in both reactors, sulphidogenesis and methanogenesis represented 50 and 50% in ER and 48 and 52% in MR, correspondingly. That represents a good equilibrium among these microbial populations reflecting an adaptation since the start of the reactors' operation. The situation was changed with lot 4 of textile wastewater when the salinity increased from 7.5 to 12.6, dropping the COD removal efficiency to 38 and 34% in ER and MR, with corresponding percentage derived to sulphidogenesis to 36 and 39%. The values for each of the five lots can be found in Table 2 of Supplementary Material.

Sulphide measured was, on average, 30.5 ± 32.7 , 95.2 ± 30.8 and 78.0 ± 24.6 mg S²⁻·L in CR, ER and MR effluent, respectively. The values of sulphide for each of the five lots and the theoretical amount of H₂S released can be found in Table 3 of Supplementary Material.

Based on the literature, the SRB have the ability to use various electron donors for growth, but polymeric organic compounds are not direct substrates for them; instead, low molecular weight substrates, such as lactate, formate, and ethanol (or hydrogen), are the compounds usually oxidized by SRB, as is the case of the common *Desulfovibrio* and *Desulfotomaculum* species (Liamleam & Annachhatre 2007; Muyzer & Stams 2008).

Molasses, which comprises a high amount of sucrose (Annachhatre & Suktrakoolvatt 2001), is first fermented by microorganisms such as lactobacilli into by-products that act as electron donors and a carbon source for SRB, such as lactate or pyruvate (Maree *et al.* 1986; Maree *et al.* 1987). *Desulfovibrio* and *Desulfomicrobium* species ferment pyruvate to form acetate, carbon dioxide and hydrogen as products. In efficient hydrogen-consuming systems (by methanogens), these microbes can also oxidize lactate and ethanol into acetate (Muyzer & Stams 2008). Conversely, ethanol alone, or as a fermentation product of the anaerobic degradation of carbohydrates, can be completely oxidized by SRB (Liamleam & Annachhatre 2007).

As degumming is commonly conducted by the textile industry, starch may be available as a carbon source, but it is not a direct substrate for SRB (Muyzer & Stams 2008). In CR, although the COD/SO₄²⁻ ratio (Table 1) was close to the stoichiometric value of 0.67, sulphate removal was very low, as previously presented. This indicates that the COD from the textile wastewater may not be easily degradable or easily available for SRB, which was confirmed by the low BOD_{5,20} values compared with those of COD (Table 1).

Regarding colour removal, apparently, the microorganisms were less dependent on the availability of an easily degradable carbon source. Removal efficiencies for CR, ER and MR were, on average, $57 \pm 20\%$, $55 \pm 21\%$, and $47 \pm 27\%$, respectively. The three reactors achieved 86% of colour removal around day 35 of the experiment (Figure 2(c)). In CR, colour removal around 61% was found on the 8th day, achieving 87% of maximum efficiency around day 40. The colour removal efficiency in CR deserves attention.

Amorim *et al.* (2013) correlated colour, measured by mg Pt-Co·L⁻¹, and the concentration of DB22, widely used in the textile industries. According to the authors, 400 mg Pt-Co·L⁻¹ corresponded to 65 mg·L⁻¹ of DB22. In the present work, if we consider that the measured colour in the influent of lot 5 (570 mg Pt-Co·L⁻¹) is derived from the dye DB22 (4 azo bonds – the worst scenario), it would correspond to 92.6 mg DB22·L⁻¹ of dye concentration. The complete removal of 1 mol of DB22 (1,083.97 g) requires 16 mol of electrons (four electrons for each azo bond), thus resulting in 1.37×10^{-3} mol·L⁻¹ of electrons required for the complete colour removal of 92.6 mg DB22·L⁻¹ present in the last lot of phase 1. Considering molecular oxygen reduction to water (4 mol of electron for 32 g of O₂), the COD required for the complete dye removal would be 10.94 mg O₂·L⁻¹, which only represents 2% of the COD measured in lot 5 (490 mg O₂·L⁻¹ – Table 1); therefore, the low COD value required for dye removal in CR justifies the good colour removal even with little or no apparent COD removal.

The use of molasses as the electron donor for colour removal is not commonly reported in the literature, while ethanol has been frequently used. Menezes *et al.* (2019), treating synthetic textile wastewater and using ethanol as the carbon source, found COD removal efficiency of 76% and DB22 removal efficiency of 81% during the steady state of their study.

Finally, regarding alkalinity and ORP behaviour, COD and sulphate removal was always linked to both parameters. In the supplemented reactors, the production of bicarbonate alkalinity (the average values during lot 3 were 1,007.0 and 1,214.4 mg CaCO₃·L⁻¹ for ER and MR, respectively) and ORP close to -400 mV were observed on almost all days of analysis. In CR, the best sulphate removal efficiency (23%) was detected around day 30 (Figure 2(b)), when the bicarbonate alkalinity increased from 489.5 to 1,183.7 mg CaCO₃·L⁻¹ and when ORP achieved -389.5 mV (Table 2).

Although ORP of around -200 mV has been cited as the standard for sulphate reduction through H₂S formation (Santos *et al.* 2007), in this study, ORP close to -400 mV was found when the treatment achieved the best removal efficiencies of COD (Figure 2(a)), sulphate (Figure 2(b)) and colour (Figure 2(c)). Similar results were found by Amaral *et al.* (2014), who obtained a methanogenic environment in a UASB reactor treating real textile wastewater (average ORP of -357 mV).

3.2.1. Salinity influence

The salinity of the 4th lot of wastewater was 12.6 g·kg⁻¹, which is about one-third of the seawater salinity (35 g·kg⁻¹) (Ibrahim & Attia 2015). The increase in salt concentration was a consequence of the high amount of sodium chloride and sodium metabisulphite, used in dyeing and bleaching processes, respectively, by the industry, which were predominantly performed among several other processes when the wastewater was generated.

The 4th lot was firstly used on the 44th day of operation and immediately caused a decrease in the removal efficiencies of the three reactors. Considering the last day of monitoring of the 3rd and the 4th lots, COD removal dropped from 79% to 0% in CR, from 63% to 40% in ER, and from 63% to 27% in MR, while for colour the corresponding values went from 87% to 22%, from 83% to 3% and from 84% to 0%; and for sulphate, the values stayed below 10% and dropped from 65% to 12% and from 52% to 14% in CR, ER and MR, respectively. During the days for lot 4, average values of the COD removed used for sulphidogenesis and methanogenesis indicated that the first was harmed the worse by the increase in salinity. Corresponding values of 36 and 64% were found in ER, and 39 and 61% in MR, out of the 38% (ER) and 34% (MR) of COD removal efficiency for the period (Table 2 of Supplementary Material). Additionally, the reactors' self-buffering capacity diminished expressively when fed with lot 4 (Table 2).

In the 5th lot, the salinity of around 6 g·kg⁻¹ relieved the osmotic pressure in the reactors. ER and MR recovered immediately; sulphate and colour removal were increased to around 50%, the production of bicarbonate alkalinity was detected again. CR did not recover as ER and MR (Figure 2, Table 2). The percentage of the COD removed used in ER increased to 54% for sulphidogenesis and decreased to 45% for methanogenesis. While in MR, the sulphidogenesis was responsible for 69% out of around 50% of COD removed against 31% for methanogenesis (Table 2 of Supplementary Material).

Regarding dye removal, the inoculum used in this study (Amaral *et al.* 2014) was well adapted to treat textile wastewater with high colour content (mix of different dyes) and relatively high salinity (up to 4.4 g·kg⁻¹). The same biomass was also used

Table 2 | Wastewater lot number, salinity (g·kg⁻¹), ORP (mV), pH range, and bicarbonate alkalinity (BA) (mg CaCO₃·L⁻¹) in the influent and in CR, ER and MR effluents during phase 1

Lot - salinity	Day	Influent		CR effluent		ER effluent		MR effluent	
		ORP	BA	ORP	BA	ORP	BA	ORP	BA
1 - 7.2	3	11.7	57.8 (321.7 ^a)	82.3	178.7	-361.4	309.9	-305.6	282.1
2 - 6.2	10	4.2	214.3	82.5	144.9	-325.5	385.7	-309.7	211.5
	18	8.0	179.3	-164.5	222.7	-387.7	495.9	-345.0	289.7
3 - 7.5	30	-24.1	489.5	-389.5	1,183.7	-403.5	1,354.3	-391.1	1,458.0
	43	-12.4	864.0	-379.4	1,237.7	-408.2	2,013.1	-402.1	2,324.2
4 - 12.6	46	-335.5	598.3	-372.2	760.3	-409.9	1,773.4	-406.5	1,913.8
	59	35.6	2,246.4	-319.2	1,737.8	-389.7	2,241.2	-402.5	2,116.4
5 - 6.2	65	-120.6	319.7	-265.3	175.0	-399.7	922.3	-398.2	868.3
	69	-22.4	324.0	-11.1	118.8	-397.8	887.8	-399.2	788.4
	73	22.7	261.6	12.5	172.2	-402.1	817.5	-398.4	797.9

^aBicarbonate alkalinity in MR influent after the addition of the buffer.

by Köchling *et al.* (2016); in their study, they found an adapted azo dye-degrading microbial community with the ability to treat a real textile wastewater under the harsh condition of salinity (up to $10 \text{ g}\cdot\text{kg}^{-1}$), which justifies the good recovery of colour removal efficiency in CR. Nonetheless, the inoculum was not able to recover the sulphate removal behaviour, as it had been previously exposed to a lower sulphate content (below $500 \text{ mg SO}_4^{2-}\cdot\text{L}^{-1}$) (Amaral *et al.* 2014) than that found in lot 5 ($1,568.6 \pm 50.8 \text{ mg SO}_4^{2-}\cdot\text{L}^{-1}$), reinforcing the dependence of SRB on favourable conditions, which was promoted in ER and MR by the addition of ethanol and molasses, respectively.

3.2.2. Temporal profiles and kinetic study

Although the reactors did not reach the steady state during our experimental period, the temporal profile performed with ER and MR gave us a better view of what was happening into both reactors during one cycle (24 h) and under two different COD/SO₄²⁻ ratios (0.67 and 1.5).

In the first profile (Figure 3) the COD/SO₄²⁻ ratio of 0.67 was the stoichiometric ratio for sulphate removal by the consumption of the external carbon source added (ethanol or molasses), without considering the COD content from the textile wastewater itself (average of $245 \text{ mg O}_2\cdot\text{L}^{-1}$ for lot 5).

The COD removal efficiencies were 63 and 69%; while sulphate removal efficiencies were 23 and 32% for ER and MR, respectively. This indicated that the sulphate reduction pathway was not dominant for organic matter removal; only 23 and 29% of the COD removed were used for sulphate reduction, in the respective reactors. Methanogens were players too, considering the ORP values found during the monitoring period (Table 2) and the low COD needed for colour removal, as presented before.

Nevertheless, in the second profile, when more ethanol or molasses were added at the ratio of 1.50, SRB found better developing conditions (98% removal for ER and 81% for MR), which may be due to a temporal accumulation of intermediate substrates. This is a consequence of higher energy conservation of fermentative microbes compared with that of the

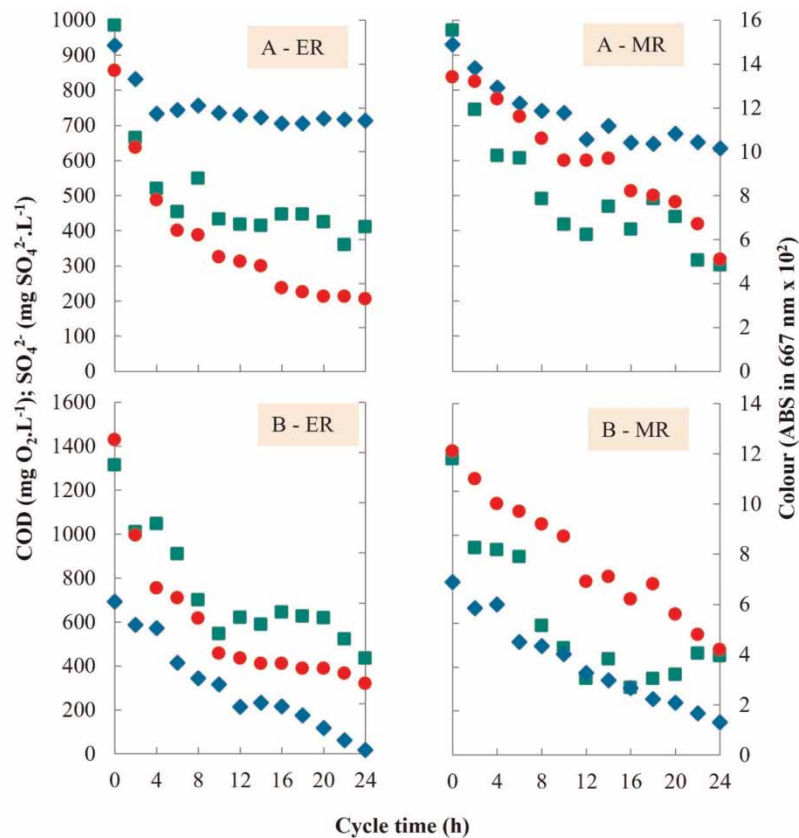


Figure 3 | Temporal profiles performed in ER and MR for COD (●), sulphate (■) and colour (values of absorbance are multiplied by 10²) (◆) during phase 1 with COD/SO₄²⁻ equal to 0.67 (a) and 1.5 (b).

methanogens (Thauer *et al.* 1977; Muyzer & Stams 2008). Out of the 67% of COD removed in both reactors (around 900 mg O₂·L⁻¹), 51% in ER and 48% in MR were used for sulphate reduction. These values are similar to those found during the period when the best performance of phase 1 (lot 3) was observed, in which sulphidogenesis and methanogenesis were in equilibrium.

Regarding the kinetics analysis of sulphate, the degradation observed only in the first hours of the cycle made it unreasonable to calculate and compare its removal rate due to an unreliable adjustment in both reactors when the COD/SO₄²⁻ ratio was 0.67. When the electron availability was increased (COD/SO₄²⁻ = 1.50), it was possible to verify that different electron sources had no influence on the removal rate (Table 3).

Conversely, colour removal was stable in both profiles with removal around 76% for ER and 63% for MR (Figure 3). A zero-order kinetic adjustment in MR suggested that the removal rates were the same under the two COD/SO₄²⁻ ratio conditions (Table 3).

Some authors have observed a first-order kinetic adjustment for the decolourization by mixed anaerobic sludge as a process dependent on the residual azo dye concentration or the electron transference, and not on the production of the reducing equivalent (van der Zee *et al.* 2001; Menezes *et al.* 2019; Oliveira *et al.* 2020). However, none of the studies has considered several electron acceptors being reduced simultaneously, as reported here, with a high amount of sulphate and a mix of various dyes.

Contrary to previous reports, our zero-order kinetic adjustments, now considering both sulphate and colour reduction rate, indicated that the production of the reducing equivalents was the limiting factor. This was reported in the case of pure cultures (van der Zee *et al.* 2001), with reactions occurring at a constant rate without dependence on electron concentration (Levenspiel 1987).

Regarding colour removal rates in the ethanol reactor, it must be considered that H⁺ production in ER occurred probably faster than in MR, due to the molasses fermentation process. Thus, rapidly available H⁺ in ER may have permitted colour degradation at a higher rate already in the first hours of the cycle, resulting again in an unreliable adjustment of zero-order kinetic in ER.

3.3. Phase 2 – monitoring of the continuous reactors

Table 4 brings ORP, bicarbonate alkalinity, and the average values of pH and dissolved oxygen through the phase 2 in both UAS and SAB reactors. During the continuous experiment, ORP variation and the average values of DO showed the environmental changes from an oxidant wastewater influent in the UAS reactor (DO = 0.43 ± 0.4 mg O₂·L⁻¹ and ORP = -5.9 ± 30.8 mV), to an anaerobic condition in its effluent (DO below the detection limit and ORP = -372.5 ± 72.9 mV); and further, returning to a less reductant condition in the SAB effluent (DO = 0.16 ± 0.1 mg O₂·L⁻¹, ORP = -115.4 ± 79.0 mV). The effluent from the UAS reactor showed pH near 7 during the entire experimental period (Table 1 of Supplementary Material).

Considering the addition of molasses as an external carbon source, the wastewater used during the second phase presented a COD/SO₄²⁻ ratio of 1.5 ± 0.2; 2.7 ± 0.3; and 3.5 ± 0.4 for lots 6, 7 and 8, respectively.

Figure 4 shows the COD, sulphate, and colour removal efficiency values found in the effluent of UAS and SAB. Considering the entire experimental period, The COD removal efficiency was about 50% in UAS during the first 20 days (ORP close to -400 mV, Table 4), whereas it reached 80% in SAB, in the same period; however, this can be attributed to the adsorption process, as the aerated biofilter was not inoculated. The average removal efficiencies for UAS were 57 ± 16% for COD,

Table 3 | Values of kinetic constant, kinetic adjustment and kinetic order applied to the removal of colour (in MR, when COD/SO₄²⁻ = 0.67 and COD/SO₄²⁻ = 1.50) and sulphate (in ER and MR when COD/SO₄²⁻ = 1.50)

	COD/SO ₄ ²⁻ = 0.67		COD/SO ₄ ²⁻ = 1.50	
	ER	MR	ER	MR
Sulphate	Not calculated due to low removal efficiencies		k ₀ = 30.66 mg·L ⁻¹ ·h ⁻¹ ; R ² = 0.9460 Zero order	k ₀ = 29.17 mg·L ⁻¹ ·h ⁻¹ ; R ² = 0.9648 Zero order
Colour	Not adjustable to 'zero order' kinetics	k ₀ = 0.0031 mg·L ⁻¹ ·h ⁻¹ ; R ² = 0.9706 Zero order	Not adjustable to 'zero order' kinetics	k ₀ = 0.0034 mg·L ⁻¹ ·h ⁻¹ ; R ² = 0.9685 Zero order

Table 4 | Wastewater lot number, salinity ($\text{g}\cdot\text{kg}^{-1}$), LAS concentration ($\text{mg}\cdot\text{L}^{-1}$), ORP (mV), bicarbonate alkalinity (BA) ($\text{mg CaCO}_3\cdot\text{L}^{-1}$), pH range and DO average values ($\text{mg O}_2\cdot\text{L}^{-1}$) in the influent, in UAS and SAB effluents during phase 2

Lot – Salinity – LAS	Days	Influent		UAS		SAB	
		ORP	BA	ORP	BA	ORP	BA
		DO 0.43 ± 0.4		BDL		0.16 ± 0.1	
		pH 6.2–7.8		6.4–8.4		7.6–8.7	
		ORP	BA	ORP	BA	ORP	BA
6 – 4.7 – <1	1	–19.7	236.2	–393.5	292.0	47.0	474.0
	6	–12.6	208.2	–398.7	560.0	–22.4	444.0
	13	–10.1	242.0	–419.1	596.0	–85.2	426.0
	22	–24.9	228	–409.1	944.0	–125.0	990.0
	28	–55.0	161.2	–423.2	1,198.0	–154.6	959.2
	36	–58.0	312.4	–426.2	1,313.4	–158.8	1,333.3
7 – 3.7 – 5.9	42	–10.3	274.0	–432.6	1,106.0	–12.2	922.0
	49	15.2	56.1	–404.4	496.0	–125.8	546.0
	57	12.3	73.3	–323.6	118.4	–226.7	310.2
	59	12.3	111.9	–162.3	65.8	–146.2	210.6
8 – 5.8 – 2.9	67	52.8	203.9	–329.7	172.3	–209.9	320.8
	71	27.1	19.8	–347.8	275.2	–165.5	455.4

BDL, below the detection limit.

48 \pm 19% for sulphate, and 26 \pm 21% for colour. While considering the entire system (measured in the effluent of SAB), efficiencies were 74 \pm 14% for COD, 37 \pm 12% for sulphate, and 26 \pm 21% for colour removal, respectively.

Highest COD removal efficiencies were achieved around day 28, reaching 94% in the final effluent of the two reactors, with ORP close to –430 mV and –200 mV in UAS and SAB effluents, respectively (Figure 4(a), Table 4). In SAB, the biomass started to self-develop around the 12th day, indicated by the decrease in ORP values (from –14 to –100 mV) because of the DO consumption by bacteria (Kato *et al.* 1997).

Regarding sulphate removal, an increase in efficiency was observed after the first month of experiment (Figure 4(b)). At around day 45, sulphate removal efficiency in UAS was 65%, reaching a maximum of 82% during the first days of lot 7. In UAS, the amount of the COD removed used for sulphidogenesis and methanogenesis achieved 43 and 57%, respectively, during the use of lot 7 (Table 4 of Supplementary Material). Sulphide measured in the anaerobic reactor stayed around 106.3 \pm 43.5 mg S²⁻·L. The values of sulphide for each of the three lots can be found in Table 5 of Supplementary Material.

In SAB, due to aeration, part of the sulphide generated in the anaerobic reactor was reoxidized to sulphate and the global efficiency of the system decreased to 50%, considering sulphate removal. Between days 30 and 45, the sulphide measured in the UAS effluent was 150 \pm 46 mg S²⁻·L⁻¹, while in the SAB effluent, it decreased to 8 \pm 17 mg S²⁻·L⁻¹. Carvalho *et al.* (2022), treating a synthetic textile wastewater containing salinity (2.84 g·kg⁻¹), DB22 (45 mg·L⁻¹), starch (1,000 mg O₂·L⁻¹), and sulphate (209 and 1,270 mg SO₄²⁻·L⁻¹), attributed sulphide oxidation in their aerated continuous stage to the futile cycle of sulphur. In the case of the anaerobic continuous stage, the authors discussed that for the chemical reduction of azo bonds, the biogenic sulphide served as an electron donor. According to Van der Zee *et al.* (2001), in an anaerobic environment rich in sulphide, the chemical reduction of an azo dye will be part of the decolourization process; thus, this has likely occurred in the present experiment, but it was not further investigated.

In contrast with observations in phase 1, colour removal efficiency reached 65% only after 13 days of experiment in UAS. In SAB, adsorption may have been a mechanism for colour removal during the first days (Figure 4(c)). This was also observed by Ferraz *et al.* (2011), treating real textile wastewater in a similar anaerobic–aerobic system.

Until the day 15, colour removal in UAS, indicated by the lower peak at 573 nm, was associated with the formation and accumulation of aromatic amines, observed in the range from 200 to 350 nm (Figure 5(a)). At the same day, the aromatic amines formed in the UAS reactor were not removed in the SAB reactor, which is another evidence that the aerobic biomass was not established yet.

Between days 28 and 45, the anaerobic reactor presented the maximum colour removal efficiency of 81% (Figure 4(c)). During the days of best global performance, the colour removal that occurred in UAS was followed by the removal of aromatic amines in SAB (Figure 5(b)). Nevertheless, despite biomass activity in SAB, part of the removal of aromatic amines

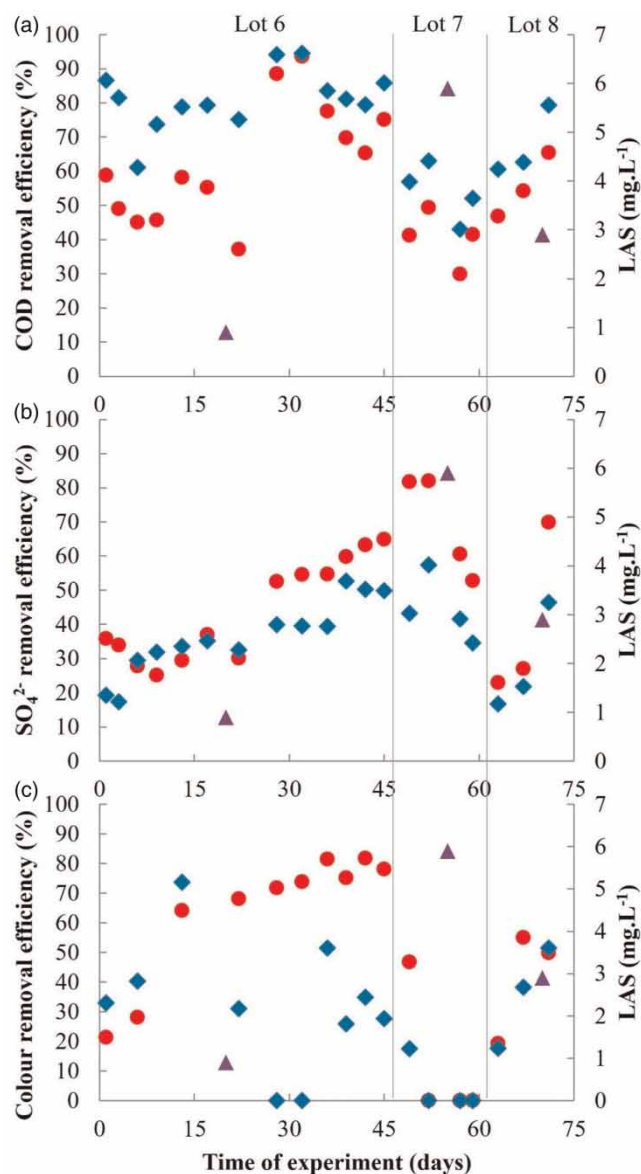


Figure 4 | Removal efficiencies of COD (a), sulphate (b) and colour (c) in UAS (●) and in SAB (◆) effluents, and LAS concentration (▲) ($\text{mg}\cdot\text{L}^{-1}$) in each lot of wastewater used in phase 2.

may be a consequence of their abiotic autoxidation, which led to a decrease in colour removal efficiency. The compounds formed from autoxidation reactions resulted in an increase in the absorbance values, with the absorbance peak close to the influent characteristic wavelength (573 nm) (Figure 5(b)). Sometimes the peak was even greater than that of the wastewater's, bringing the colour removal efficiency equal to zero (Figure 4(c), days 28 and 32). This was observed in spectrophotometric scans of many days of monitoring (data not shown).

In some studies using a synthetic substrate, the same decrease in colour removal efficiency was observed in the aerobic stage of a similar system (anaerobic–aerobic stages) for the treatment of dyes (van der Zee *et al.* 2001; Jonstrup *et al.* 2011; Menezes *et al.* 2019; Carvalho *et al.* 2022). This can be explained by the tendency of some azo dyes present for autoxidation when exposed to aerobic conditions, forming coloured polymeric structures that are recalcitrant for biological treatment (Jonstrup *et al.* 2011).

Considering a real textile wastewater, some previous studies that applied a continuous anaerobic–aerobic system have also found comparable results in terms of removal efficiencies. Ferraz *et al.* (2011) achieved 77 and 86% of global removal

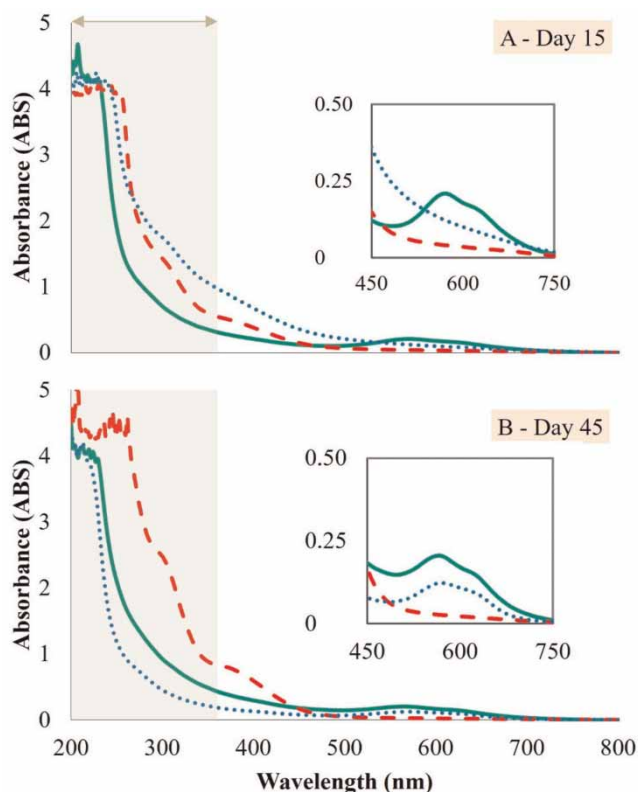


Figure 5 | Spectrophotometric scans of the real wastewater influent (—) and effluents from UAS (---) and SAB (.....) on days 15 (a) and 45 (b) of operation during phase 2.

efficiencies of COD and colour, respectively, when HRT was 24 h. The anaerobic reactor represented 59 and 64% of removal of the same parameters. Contrarily to our findings, these authors did not mention any decrease in colour removal efficiency when the anaerobic effluent was exposed to oxygen. Regarding sulphate removal, the study cited reached efficiencies between 14 and 63%, but the concentration of the compound was much lower (between 14.5 and 52.0 mg $\text{SO}_4^{2-} \cdot \text{L}^{-1}$) than the concentrations found in the present study.

Amaral *et al.* (2014) obtained an average COD removal efficiency between 56 and 71% for the entire system. Despite the low average removal efficiencies of sulphate (varying from 41 to 54%) in the anaerobic reactor, the authors reported that there were sufficient electrons for the complete removal of the compound (varying from 269 to 464 mg $\text{SO}_4^{2-} \cdot \text{L}^{-1}$), indicated by the COD/ SO_4^{2-} ratio between 2.4 and 3.0. In addition, as we found in our study, they observed sulphide oxidation under aerobic conditions.

In the two studies cited above, the presence of salts was mentioned as a possible interference for biological treatment efficiency. The values of salinity varied from around 1.6 to 19 g·kg⁻¹ at first (Ferraz *et al.* 2011), and they were up to 4.4 g·kg⁻¹ in the second work (Amaral *et al.* 2014). In both studies, HRT was varied but no electron supplementation was performed; and high sulphate content impaired colour removal under anaerobic conditions. This occurrence differed from the findings of our study, maybe due to a prioritized use of electrons for sulphate reduction in their experiments.

Using a similar up-flow anaerobic structured-bed reactor similar to ours and HRT of 24 h, Florêncio *et al.* (2021) treated a synthetic wastewater containing azo dye and ethanol as the energy source. With a COD/ SO_4^{2-} ratio equal to 5 and a sulphate concentration of 48 mg $\text{SO}_4^{2-} \cdot \text{L}^{-1}$, average removal efficiencies were 78, 77 and 68% for COD, sulphate and colour, respectively. The authors highlighted the good performances for colour and organic matter removal in the presence of sulphate ions and stated that sulphidogenesis did not outcompete methanogenesis nor significantly impaired reductive decolorization.

In our study, we did not observe any evidence of competition between dye-reducing and SRB even with a high content of sulphate (>500 $\text{SO}_4^{2-} \cdot \text{mg} \cdot \text{L}^{-1}$). By contrast, dye reduction occurred simultaneously with sulphate reduction. This may be

explained by the supplementation of an easily biodegradable organic matter (molasses) and the low electron demand for dye reduction, as discussed previously.

Using only a UASB reactor, Somasiri *et al.* (2008) achieved colour and COD removal efficiency of around 97 and 90%, respectively, during a 24-h HRT and with no carbon supplementation. The authors called for attention to the necessity of a degradable carbon and energy source for microbes, and they suggested a mixing of textile with food factory or municipal wastewater for low organic load before feeding the anaerobic reactor.

Based on these previous works and in our results, it is possible to infer first that a low sulphate and salt contents, aligned to an available organic matter which is easily biodegradable, is fundamental to provide a more favourable condition for a biological anaerobic-aerobic treatment system; and second, that a better control of the oxidation reactions under aerobic conditions (second stage of this treatment strategy) is crucial.

3.3.1. Influence of LAS

On day 49, excessive foaming was observed in the upper part of the anaerobic reactor. LAS analysis revealed a concentration of 5.9 mg LAS·L⁻¹ in the wastewater (lot 7), around six times greater than the values of the previous lots (always lower than 1 mg·L⁻¹) (Table 1).

Changes in the wastewater constituents may cause a disturbance in the anaerobic reactor and, consequently, in the entire system. Souza *et al.* (2016) analyzed methanogenesis inhibition caused by different levels of LAS present in domestic wastewater and found a reduction of 30% in methanogenesis activity when the LAS concentration was increased from 0 to 10 mg LAS·L⁻¹, and 50% when the surfactant concentration was 30 mg LAS·L⁻¹.

In our study, the anaerobic biomass had never faced a LAS concentration higher than 1 mg LAS·L⁻¹; thus, a small increase in the surfactant content caused the observed disturbance. During the use of lot 7, between days 49 and 59, considering the anaerobic reactor, ORP reached -162.3 mV, the alkalinity was consumed (Table 4), pH dropped to the lowest value of 6.4, and COD, sulphate, and colour removal efficiencies decreased to 30, 53 and 0%, respectively (Figure 4).

Lot 7 was then replaced by lot 8, which presented a LAS concentration again higher than the usual (2.9 mg LAS·L⁻¹). Although the production of bicarbonate alkalinity did not exceed 300 mg CaCO₃·L⁻¹ (Table 4), the system indicated a recovery when considering COD and colour removal efficiencies already on the first day of analysis. An increase of sulphate removal efficiency was observed only on the last day of lot 8 (Figure 4), which is in accordance with the 15% of COD removed used for sulphidogenesis against 85% for methanogenesis (Table 4 of Supplementary Material). This indicates that the SRB activity was again worse impaired and that they would probably need more time to re-establish themselves in the UAS behaviour. The values of ORP were close to -350 mV (Table 4), similar to those observed by Amaral *et al.* (2014) (-357 mV on average) and still within the range for azo dye reduction, from -100 to -500 mV (Santos Cervantes & van Lier 2007).

3.4. General aspects of continuous and batch reactors' performance

In the two phases of this study, the best removal efficiencies of COD, colour and sulphate under the anaerobic condition were related to values of ORP around -400 mV and the production of bicarbonate alkalinity above the value of 300 mg CaCO₃·L⁻¹. This concentration of bicarbonate alkalinity is the recommended value for self-sufficiency of anaerobic treatment of domestic wastewater (Metcalf & Eddy 2003), which is comparable with the textile wastewater treated in our study in terms of low organic load.

Yet considering anaerobic reactors, when a continuous system was applied (phase 2), the treatment achieved COD and sulphate maximum removal efficiencies were greater than the corresponding values found for the batch system (phase 1, MR). In the UAS reactor, the maximum COD and sulphate removal efficiencies were 94 and 82%, compared with 68 and 70% in MR, respectively. In addition to the higher salinity presented by the lots used during phase 1, fresh wastewater with harsh compositions added into the system every day, with an exchange volume of 50% may have caused a daily impact to the microbial community.

Another important fact is the sulphide accumulation in the batch reactor during its cycle time, which may have inhibited bacterial activity at some level. Visser (1995) suggested anaerobic filter reactors can tolerate higher concentration of sulphide than reactors with suspended sludge. Chen *et al.* (2008) bring different aspects related to sulphide toxicity, including the total and unionized forms of the compound, their concentration in the environment, pH range, among others. The authors emphasize that different levels of sulphide can cause inhibition to several trophic groups, in different anaerobic degradation steps.

Additionally, they suggest that in reactors with fixed biomass, methane-producing microorganisms (MPM) can better tolerate sulphide due to an adaptation to free H_2S .

In our study, under the continuous regime (phase 2), the constant gas (unionized form, H_2S) and effluent outflow (ionized form as HS^-) against the exchangeable volume of 50% in batch reactors (phase 1) may have softened the toxicity generated by the sulphur compounds, resulting in a more favourable environment for the microbial community.

With regards to the COD used by SRB and MPM, Visser (1995) found that SRB and MPM can fairly compete under pH values between 6.9 and 7.7, by performing the same range growth rate and being equally inhibited by sulphide. Indeed, in the present work, during the days of best performance of both systems, the organic matter removed through sulphidogenesis and methanogenesis was around 50% each (in ER and MR, lot 3, and in UAS during the last days of lot 6 and first days of lot 7) (Tables 1, 2 and 4 of Supplementary Material).

Nevertheless, the sulphide measured in the anaerobic reactors (lower than $150 \text{ mg S}^{2-} \cdot \text{L}$ during the entire experimental period, in both phases 1 and 2) was not in a inhibition range in comparison to the salinity and the LAS contents. The microbial community was hindered by these two wastewater constituents, mainly the SRB group, which was able to remove less amount of COD when both batch and continuous systems suffered the impact of the respective interfering compounds.

Finally, apart from all these circumstances, the short period of feeding the reactors with each lot of wastewater, with fluctuating composition, prevented the microbes to establish themselves under such conditions, and thus the reactors to reach the steady-state. In this study, the goal was to evaluate the biological treatment of a real wastewater with minimum action to strengthen the microbial community, for instance the supplementation of phosphorous, which represent an increase in the cost of treatment.

4. CONCLUSIONS

The supplementation of an external carbon source improved the performance of the anaerobic reactors used to treat a real textile wastewater rich in sulphate, salinity, and colour by providing a more friendly characteristic to the influent wastewater. The addition of ethanol and molasses had no influence on dye transformations, but it improved sulphate reduction, being necessary when both dye and sulphate removal were targeted along with organic matter removal. Furthermore, the addition of molasses or ethanol gave robustness to the reactors, which quickly recovered from an adverse increase in salinity (from 7.5 to $12.6 \text{ g} \cdot \text{kg}^{-1}$).

Aromatic amines and sulphide were formed as a result of dye and sulphate reduction in the anaerobic reactor, respectively. Nevertheless, the oxidation of these compounds needs to be better controlled in the aerobic stage, since it is directly related to the overall efficiency of the treatment.

Finally, aiming to achieve an efficient and stable biological treatment, we propose that the textile wastewater must be monitored and managed before being sent to the reactors. In order to treat a less hostile influent in the reactors (lower sulphate values, availability of an easily degradable organic material and fewer interferences, such as salt and surfactants), the wastewater from different predominant industrial processes should be mixed in a way to minimize the high concentration of the interferences. Furthermore, the supplementation with an external carbon and electron source, easily biodegradable, must be considered as a strategy to achieve high treatment efficiency.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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