Effect of starch and ethanol as electron donors, and sulfate on the reductive decolourisation of azo dye Direct Black 22

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Abstract
Textile wastewater contains organic matter, dyes, sulfate, salt and other chemical constituents. Azo dyes are the most used colorant and have a great variety of molecules. When released into the environment, those effluents and their degradation products can cause environmental pollution and risks to the public health. Therefore, studies are required for an efficient treatment plant design and operation. In this study, batch experiments were carried out to evaluate the influence of two electron donors (starch and ethanol) and sulfate presence (0 and 250 mg.L-1) in the reduction of the dye Direct Black 22 (DB22). When starch was present, azo reduction started immediately but it was not coupled with sulfate reduction. In the presence of ethanol, DB22 and sulfate were reduced simultaneously. The complete sulfate reduction occurred only when propionate and butyrate were above 100 mg L^{-1}. In all experiments, there was VFA production, mainly acetate. When sulfate was added, the first-rate constant of decolourisation (*k*) increased 1.5 and 2.4 times for starch and ethanol, respectively, compared with those without addition. When sulfate was not added and ethanol was the electron donor, high acetate concentrations (1000 $mg.L^{-1}$) were observed. Moreover, the presence of sulfate in the bulking liquid considerably increased the color removal rate for both electron donors.

Keywords
Microbial decolorization; azo dye; sulfate reduction; aromatic amines; VFA production.

INTRODUCTION

The textile effluent is considered as extremely complex because different chemicals are introduced during the industrial process. The physical-chemical technologies available for the treatment of the final effluent, like adsorption, reverse osmosis, filtration or ozone oxidation, are still expensive for many industries, mainly for those of small and medium sizes. That is why there has been increasing interest on the use of biological treatment along the recent years (Van der Zee and Cervantes, 2009; Spagni et al., 2012). It is known that the combination of anaerobic and aerobic steps is required to promote the complete mineralization of the azo dyes usually found in textile wastewaters (Ferraz Jr. et al., 2011; Yasar et al., 2012). Nevertheless, the azo dye anaerobic degradation still requires studies due to the large range of dyes commercially available. Consequently, they can significantly influence the wastewater composition and therefore, further research on their pathways degradation should be conducted. Another problem is the sulfate present at high concentrations in textile effluents because of the sodium metabisulfite, sulfide and dithionite used during the industrial process. The sulfate redox conditions can interfere by impairing the metabolic azo dye degradation. Additionally, the electron donor source for azo dye reduction can also be determinant on the degradation rates and pathways.

The state of Pernambuco in Brazil is responsible for 15% of the national jeans production (ABIT, 2011). The laundering jeans comprises the steps of degumming, dyeing and washing, resulting in large amount of effluent rich in starch and azo dyes that are discharged in the environment. In this work, the effect of two different electron donors (starch and ethanol) and sulfate on the anaerobic degradation of the azo dye Direct Black 22, was evaluated using batch essays.

MATERIALS AND METHODS

Since the tetra-azo dye Direct Black 22 (C. I. 35435; CAS 6473-13-8) is the mostly used dye in the laundering jeans industries in Pernambuco (Ferraz Jr. et al., 2011), it was used in the synthetic wastewater prepared for this work. The molecular weight of the dye ($C_{44}H_{32}N_{13}Na_3O_{11}S_3$ -Figure 1) is 1083.97 g mol^{-1}. A COD of 685 mg O_2 $L^{-1}$was obtained for each 1000 mg L^{-1} of the dye solution that was used after the hydrolysis process, as recommended by the manufacturer (pH adjustment to 11.0 ± 0.05 with NaOH 20%; 1 hour of heating at 80°C and pH readjustment to 7.0±0.05 using HCl).

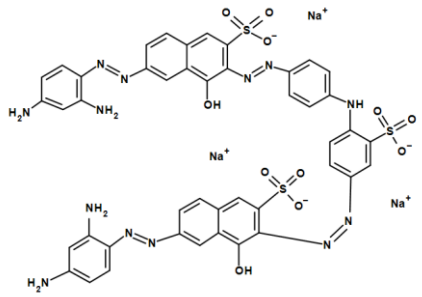

Figure 1. Chemical structure of the azo dye Direct Black 22 (C. I. 35435)

Experimental set-up and basal media

Batch experiments were conducted using three 6.80-L acrylic reactors, each filled with 5.5 L of substrate and with sludge from a UASB reactor (Ferraz Jr. et al. 2011) resulting in 1.5 g VSS.L^{-1}. All assays were incubated under agitation (60 rpm) at 30 ºC in the dark. Anaerobic conditions were established by flushing the headspace with N_2/CO_2 (80%/20%). In the substrate composition, starch and ethanol were used individually as electron donor (COD of 1300 mg O_2.L^{-1}) for the reduction of DB22 azo dye (0.06 mM). Sodium bicarbonate was used as buffer (1300 mg.L^{-1} of $NaHCO_3$). The macro- and micronutrients solution was prepared according with Florencio et al. (1993), except for the source of magnesium ($MgSO_4.7H_2O$) that was replaced by hydrated magnesium chloride ($MgCl_2.6H_2O$), in order to keep the magnesium concentration level, but without an unwanted extra source of sulfate. Four reactors were operated using starch (S) and ethanol (E) as electron donors, without (R0S and R0E) and with (R250S and R250E) external sulfate source (250 mg SO_4.L^{-1}) added as sodium sulfate.

Analytical Methods

Color removal was monitored by measuring it photometrically at the wavelength of maximum absorbance for DB22 (476 nm). Samples were centrifuged (13000 rpm, 5 min) and diluted in a phosphate buffer (9.61 g.L^{-1} NaH_2PO_4; 4.78 g.L^{-1} Na_2HPO_4 and 0.2 g.L^{-1} ascorbic acid) to prevent auto-oxidation reactions. Dye concentration was calculated from a calibration curve. COD, pH and sulfate were measured according to the Standard Methods (APHA, 2005). Volatile fatty acids (VFAs) were measured with a gas chromatograph (Agilent GC-FID) using H_2 as a carrier gas, a detector temperature at 300 ºC and a Thermo TR-WAX column (30 m x 250 µm x 0.25 µm).

RESULTS AND DISCUSSION

The first order kinetic model fitted well the dye reduction experimental data (Table 1). When sulfate concentrations were low, there were no differences between the electron donors (R0S and R0E) for the kinetic parameters. Yet with sulfate addition, the kinetic parameters obtained for dye reduction using ethanol (R250E) as electron donors were 1.6 higher than that obtained for starch (R250S), although a lag phase was observed for reactors with ethanol (Figure 2b). Considering that the seed sludge was not adapted to ethanol, this substrate was the best electron donor for dye reduction process. Nevertheless, there was no significant statistical difference ($p = 0.2362$) in the dye removal efficiencies between the electron donors, with and without sulfate.

Table 1. DB22 dye removal efficiency and kinetic parameters obtained for dye reduction for the electron donors starch and ethanol

Reactor	Sulfate addition ($mg.L^{-1}$)	Electron donor	COD removal (%)	DB22 removal (%)	k (d^{-1})	R^2
R0S	0	Starch	78.8	89.6	0.16	0.9938
R250S	250	Starch	79.6	85.9	0.24	0.9810
R0E	0	Ethanol	53.8	90.1	0.16	0.9857
R250E	250	Ethanol	85.3	89.4	0.38	0.9709

However, the dye decay rates *(k*) were 1.5 and 2.4 higher in the reactors with sulfate, R250S and R250E, than for those in the reactors without sulfate, R0S and R0E, respectively. Cervantes et al. (2007) also observed an increase in the values of *k* for azo dyes reduction when using sulfate; however, the concentrations were much higher (5 and 10 g L^{-1}) and they needed the use of redox mediators.

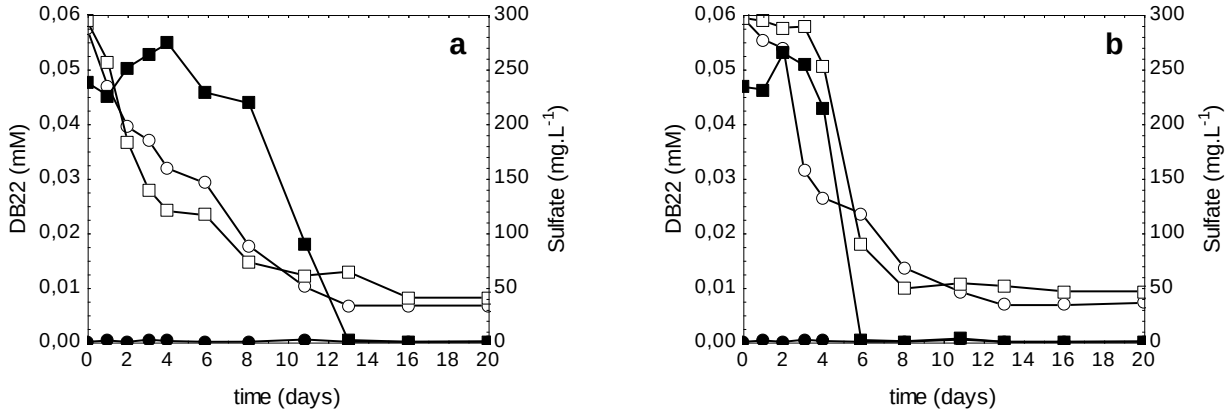

Figure 2. Decolourisation of DB22 (open symbols) and sulfate concentration (full symbols). a: Reactors with starch as electron donor. b: Reactors with ethanol as electron donor. Circles: reactors incubated without sulfate; squares: reactors with sulfate addition (250 $mg.L^{-1}$).

Figure 2 presents the evolution of DB22 and sulfate removal in all the reactors. In both starch reactors (R0S and R250S), the color degradation started immediately; however in R250S, it was not coupled with the sulfate reduction. In this reactor, the sulfate was reduced only after preferred substrates for the SRB, propionate and butyrate, became available, as shown in Figure 2a.

When the dye concentration reached 0.015 mM and propionic and butyric acids are above the 100 mg L^{-1}, sulfate is completely reduced (Figures 2a and 3a). Actually, starch and other organic polymeric compounds are not used by the SBR. In contrast, ethanol can be used directly and partially oxidized to acetate (Muyzer and Stams, 2008). Propionate and butyrate are also usual substrates for the SRB.

Since the sludge was not adapted to ethanol, a lag phase occurred for color removal in both reactors (R0E and R250E). After that period, however, the presence of sulfate in the bulking considerably increased the color removal rate (Table 1). Moreover, acetate accumulation was lower (Figure 3b) than in the reactor deprived of sulfate (R0E). When sulfate was not added and ethanol was the electron donor, high acetate concentrations (1000 mg L^{-1}) were observed. As there was an excess of electron donors in the reactors, it was not possible to observe the competition between the DB22 and sulfate which were both reduced simultaneously. By the end of the experiment (Table 1), COD removal was about 79% for both starch reactors. However in ethanol reactors without sulfate addition, the COD removal was low (54%) and acetate was the main acid accumulated. In the presence of sulfate, COD removal reached 85.3% and the VFA concentrations were below than 100 mg L^{-1}.

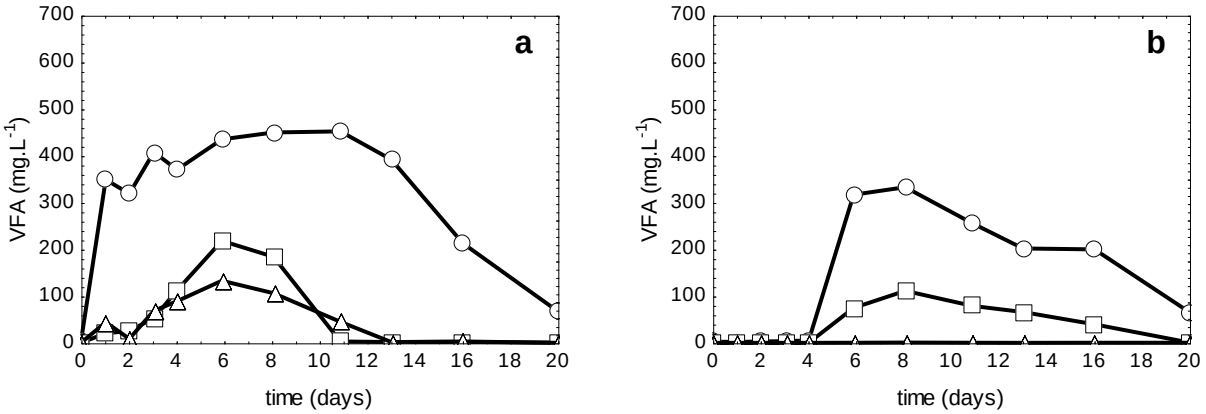

Figure 3. Volatile fatty acids accumulation observed during decolourisation of DB22. Reactors with sulfate addition in the presence of starch (a) and ethanol (b) as electron donors. Acids showed: acetate (○), propionate (□) and butyrate (Δ).

CONCLUSIONS

The effect of starch and ethanol as electron donors was evaluated on the reductive decolourisation of azo dye Direct Black 22, in the absence and presence of sulfate. When starch was present, the colour degradation started immediately but it was not coupled with sulfate reduction. Only when propinate and butyrate were above 100 mg L^{-1}, sulfate was completely reduced. The first-order dye degradation rate for starch in the absence and presence of sulfate was 0.16 d^{-1} and 0.24 d^{-1}, respectively. In all experiments, VFA was produced, mainly as acetate. When sulfate was not added and ethanol was the electron donor, high acetate concentrations (1000 mg L^{-1}) were observed. Moreover, the presence of sulfate in the bulking liquid considerably increased the color removal rate for both electron donors.

ACKNOWLEDGEMENTS

This work was supported by the National Council of Research (CNPq) and Pernambuco Research Foundation (FACEPE).

REFERENCES

ABIT – Brazilian Manufacturing and Textile Industry Association. Sector Profile. Available at: http://www.abit.org.br/. Accessed on: April 05, 2011. (in Portuguese).

APHA, AWWA, WEF (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington DC, USA: American Water Works Association/ Water Environment Federation.

Cervantes, F. J., Enríquez, J. E., Galindo-Petatán, E., Arvayo, H., Razo-Flores, E., Field, J. A. (2007) Biogenic sulphide plays a major role on the riboflavin-mediated decolourisation of azo dyes under sulphate-reducing conditions. *Chemosphere*. 68, 1082.

Ferraz, A. D. N., Kato, M. T., Florencio, L., Gavazza, S. (2011). Textile effluent treatment in a UASB reactor followed by submerged aerated biofiltration. Water Science and Technology. 64 (8), 1581.

Muyzer, G., Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. Nature reviews: microbiology. 6, 441.

Spagni, A., Casu, S., Grilli, S. (2012). Decolourisation of textile wastewater in a submerged anaerobic membrane bioreactor. Bioresource Technology. 117, 180.

Van der Zee, F. P., Cervantes, F. J. (2009). Impact and application of electron shuttles on the redox (bio)transformation of contaminants: A review. Biotechnology Advances. 27 (3), 256.

Yasar, S., Cirik, K., Cinar, O. (2012). The effect of cyclic anaerobic–aerobic conditions on biodegradation of azo dyes. Bioprocess and Biosystems Engineering. 35, 449.