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INFLUENCE OF THE APPLIED ORGANIC LOAD (OLR) ON TEXTILE WASTEWATER TREATMENT USING SUBMERGED ANAEROBIC MEMBRANE BIOREACTORS (SAMBR) IN THE PRESENCE OF REDOX MEDIATOR AND POWDERED ACTIVATED CARBON (PAC)

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Abstract - This paper evaluated the treatment of textile wastewater in submerged anaerobic membrane bioreactors (SAMBR) operated on a bench scale. Particularly, the influence of operational conditions, such as the applied organic rate (OLR) and the dilution factor (for toxicity attenuation) of the textile wastewater, was evaluated on color and organic matter removal. In addition, the effect of powdered activated carbon (PAC) on SAMBR-1 and the addition of yeast extract (source of the redox mediator riboflavin) were also investigated. The results showed that reducing the textile wastewater dilution factor from 10× (phase 1) to 4× (phase 2) and 0× (no dilution) decreased the COD and color removal efficiencies in both SAMBRs, probably due to an increase in the toxic load. Nevertheless, PAC adsorbed toxic compounds found in the textile wastewater and helped biomass acclimatization, which led to higher COD and color removal efficiencies in SAMBR-1. The presence of yeast extract enhanced color removal efficiencies 3-fold in both SAMBRs when they were fed with undiluted textile wastewater.
Keywords: Anaerobic treatment; Textile influent; Powdered activated carbon; Toxicity.

INTRODUCTION AND RELEVANCE

During their productive processes, textile industries consume large quantities of water and thereby generates high amounts of wastewater. Textile effluents are characterized by being impactful to the environment, exhibiting high color and moderate biodegradability due to the presence of dyes that did not attach to the fibers during the dying process (Kuns and Peralta-Zamora, 2002). In general, textile wastewater has a great amount of organic compounds such as starch, surfactants, softeners and fixers, as well as inorganic species such as sulfate, chloride and sodium. These contaminants may cause unpleasant aesthetic aspects; increase biochemical oxygen demand; compromise algal photosynthetic activity;

*To whom correspondence should be addressed reduce the amount of dissolved oxygen (DO); as well as cause aquatic toxicity and mortality of aquatic species (Weisburger, 2002). In addition, textile dyes damage the environment and pose a threat to human health, since some of them or their byproducts are known carcinogenic or mutagenic compounds (Kalyuzhnyi and Sklyar, 2000). It is estimated that there are more than 3,000 types of dyes and that the azo type, characterized by the presence of one or more –N=N- bond, represents ~70% of the colorants commercialized worldwide (Hunger *et al*., 2004).

In order to provide an efficient organic matter and color removal from textile effluents, different techniques (physical, chemical and biological) have been studied and applied on pilot and full scale (Firmino *et al*., 2011; Teixeira *et al*., 2014 and Lambrecht *et al*., 2015). Since each of these processes has technical or economic limitations, there is no single technology capable of efficiently and economically removing color from textile effluents (Van Der Zee and Villaverde, 2005), which keeps open research opportunities in this field.

In Brazil most textile industries employ the well-known biological process of activated sludge for wastewater treatment. This process is normally efficient in removing the organic load from wastewater, but is not so effective in decolorizing it. Activated carbon or coagulants can be used as complements, which normally generates a high amount of sludge that needs to be properly handled (Santos *et al*., 2009). An important wastewater treatment technology that has been researched for azo dye removal is anaerobic digestion (Georgiou and Aivasidis, 2006). Some electrophilic contaminants, such as azo dyes, can be used as final electron acceptors in anaerobic environments, thereby leading to the reduction of the chromophore system and color removal. However, when textile effluents have others electron acceptors such as sulfates (SO_4^{-2}), nitrate (NO_3^-) and nitrite (NO_2^-), the anaerobic process might be compromised.

Some favorable characteristics of the anaerobic system, such as low cost, operational simplicity and low sludge production, make this technology very attractive, especially in tropical and developing countries like Brazil. However, some operational problems related to textile effluent characteristics (e.g., high salinity, adverse pH value, organic and hydraulic shock loads) can contribute to lower the anaerobic microorganism growth rate, cause sludge deflocculation or granulation troubles and biomass loss. In order to minimize these effects, alternatives such as the combination of ultrafiltration membranes and powdered activated carbon (PAC) have been suggested in the literature (Hu and Stuckey, 2006; Baeta *et al*. 2013). The use of membranes immersed in anaerobic reactors increases the cell retention time and produces an effluent of better quality, which can be reused as process water (Hu and Stuckey, 2006). In turn, the addition of PAC can control toxicity, improve the granulation process and help in controlling membrane fouling due to the adsorption of proteins, biopolymers and soluble microbial products (Akram and Stuckey, 2008; Aquino *et al*., 2006).

Some authors such as Dos Santos *et al.* (2007, 2004) and Baeta *et al.* (2012) confirmed that anaerobic treatment is efficient for treating textile wastewater, especially in removing color. Dos Santos *et al* (2007, 2004) fed an anaerobic reactor with synthetic effluent containing a model azo dye, adopting a hydraulic retention time (HRT) of 24 hours, and verified high color removal efficiencies. Baeta *et al* (2012) fed a submerged anaerobic membrane reactor with real textile effluent (dilution rate of 10) employing a HRT of 24 h and the color removal efficiencies varied from 86 to 94%. Despite the high color removal efficiencies, in both cases the operational conditions are far from what would be employed in full scale conditions. It is necessary to carry out more studies to demonstrate how the SAMBR would behave when exposed to stressful conditions of organic and hydraulic shock loads.

Therefore, the main objective of this paper was to evaluate the behavior of a bench scale submerged anaerobic membrane bioreactor (containing or not powdered activated carbon in its interior) fed with real textile effluent when submitted to different HRT and organic loads.

MATERIALS AND METHODS

Apparatus and Operational Conditions

Two SAMBR (anaerobic reactors with microfiltration module placed inside of the settler compartment, as seen in Figure 1) were built using polyvinyl chloride (PVC) pipes and fittings, and had a working volume 3.25 L. The microfiltration membranes (0.8 m^2 of hollow fibers, with nominal pore size of 0.4 μm) were made of polyimide and the modules manufactured with PAM membranes (PAM – Membranas Seletivas®). During this work the reactor SAMBR-1 was operated with 4.0 $g.L^{-1}$ of PAC (Synth®) in its interior (single addition of 13 g PAC at the beginning of operation), while the SAMBR-2 was operated as control, i.e., without such adsorbent. The choice of this PAC concentration was based on work reported in the literature (Akram and Stuckey, 2008; van der Zee *et al*., 2003). Both reactors were operated at the same time (in parallel) during six operational phases (Table 1), characterized by different HRT and dilution factors (DF). Yeast extract (a cheap source of riboflavin, a known redox mediator) was added in the first four phases to enhance color removal, as previously demonstrated by Baeta *et al*. (2013). During the operation of the reactors the feed solution was kept under refrigeration at a temperature of 4 °C. The textile wastewater used to feed the reactors was collected from a textile industry located in the city of Itabirito (Minas Gerais state, Brazil).

The reactors were incubated with 0.8 L of anaerobic sludge (10 g L^{-1}) from a demo scale UASB reactor fed with raw sewage in operation at the Center for Research and Training in Sanitation (CePTS) UFMG/COPASA. This facility is located at the Arrudas WWTP, which receives the major part of the domestic sewage from the urban area of Belo Horizonte (Minas Gerais state, Brazil). During all six operational phases the temperature was maintained at 35 °C and the pH was kept in the 6.5 – 7.5 range by adding 0.1 M Na_2CO_3 or 0.01 M HCl directly into the reactors. Each phase lasted ~30 days, which was counted only after reactor stabilization. The reactors were deemed stable when the changes in COD removal efficiency were lower than 5%.

Analytical Techniques

During reactor operation, samples of influent and effluent were collected five times a week for analysis of chemical oxygen demand (COD), color, pH and turbidity. In addition, samples of anaerobic effluent and from inside the reactors (SAMBR-1 and SAMBR-2) were collected once a week for analysis of volatile suspended solids (VSS) and volatile fatty acids (VFA). The analysis of COD, pH, turbidity and VSS followed the procedures detailed in the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

COD analysis was employed with the supernatant obtained after centrifuging the sample for 15 min at 5,000 rpm (Fanem Excelsa 11 centrifuge, model 206 BL), hence the results of COD refer to the soluble fraction. For color analysis, a Dell colorimeter previously calibrated with Pt/Co standards was used. Hence, the results are given in color units (CU) which are equivalent to Hazen units.

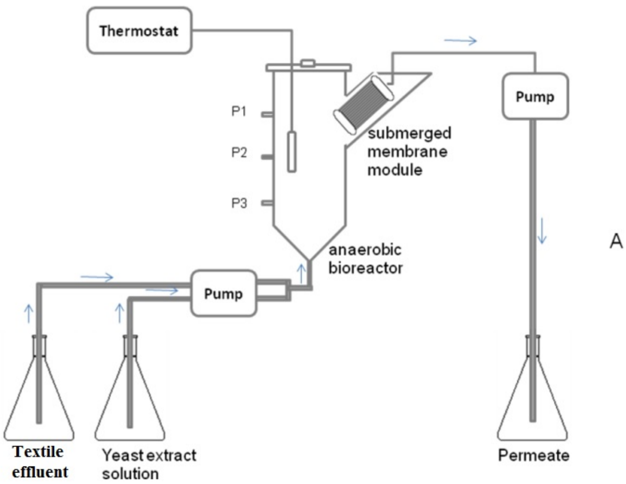

Figure 1: Scheme of the bench scale SAMBR reactors used.

Table 1: Operational phases of reactors SAMBR-1 and SAMBR-2.

Phase	HRT (h)	Dilution Factor	COD (mg.L^{-1})	*OLR (mgCOD.L^{-1}.h^{-1})	**VSS SAMBR-1 (mgVSS.L^{-1})	**VSS SAMBR-2 (mgVSS.L^{-1})	Average conductivity (mS.cm^{-1})	***SLR SAMBR-1 (kgCOD.kg VSS^{-1}.d^{-1})	***SLR SAMBR-2 (kgCOD.kg VSS^{-1}.d^{-1})	Yeast extract (mg.L^{-1})
1	24	10 ×	670	27.92	14.460	14.769	1.57	0.046	0.045	500
2	24	4 ×	1.400	58.33	20.000	17.230	4.88	0.070	0.081	500
3	12	4 ×	2.105	175.42	23.100	20.615	4.23	0.182	0.204	500
4	24	0 ×	2.100	87.5	25.846	22.461	9.98	0.081	0.093	500
5	24	0 ×	950	39.58	26.769	24.000	9.12	0.035	0.040	-
6	24	1.7 ×	400	16.67	28.923	25.846	7.85	0.014	0.015	-

*Applied organic loads; ** Median VSS concentration inside the reactors, *** Sludge loading rates calculated considering the median VSS inside the reactors.

VFA was analyzed by high performance liquid chromatography (HPLC) using an ion exchange column (Aminex HPX-87H, BioRad) which was kept at 55 °C under isocratic mode (0.6 ml min^{-1} of 0.01 M H_2SO_4). For this, 10 µL of the centrifuged samples was injected in the Shimadzu HPLC and the separated VFA detected at 210 nm with a diode array detector (DAD). The VFA method was properly validated as detailed elsewhere (Mesquita *et al.*, 2013). VFA concentration was then used to estimate the fraction of COD due to such intermediate compounds, according to Equation (1).

$$\begin{aligned}\mathrm{COD(VFA)} = {} & 0.35\mathrm{x}[\mathrm{formate}] + 1.07\mathrm{x}[\mathrm{acetate}] \\ & +1.51[\mathrm{propionate}] \\ & +1.82[\mathrm{butyrate} + \mathrm{isobutyrate}] \\ & +2.04[\mathrm{valerate} + \mathrm{isovalerate}]\end{aligned} \quad (1)$$

Statistical Analysis

In order to verify whether the differences observed between SAMBR-1 and SAMBR-2 were significant, statistical tests were employed by means of the software Statistica. The Shapiro and Wilk test was used to confirm that the results did not follow a normal distribution, and then non-parametric tests (Kruskal-Wallis ANOVA, Student- Newman and Mann-Whitney) were applied. A p-value lower than 0.05 was adopted for rejecting the null hypothesis (H_o).

RESULTS AND DISCUSSION

Effect of the Dilution Factor (DF) and Hydraulic Retention Time (HTR) on Organic Matter Removal

Figure 2 shows the influence of the DF in the textile wastewater and hydraulic retention time (HRT) on COD removal in the reactors SAMBR-1 (4.0 g/L PAC) and SAMBR-2 (without PAC) during all operational phases. By comparing the median COD removal efficiency of SAMBR-1 of 88.6%, 51.6% and 43.3% in phases 1 (DF of 10×), 2 (DF of 4×) and 4 (no dilution), respectively and of SAMBR-2 of 78.3%, 50.7% and 44.9% in the respective phases 1, 2 and 4, it is possible to see that the decrease in DF caused a reduction in the SAMBR performance. In addition, it is possible to see that in these phases the COD removal efficiencies were higher in SAMBR-1 when compared to SAMBR-2, indicating that the addition of PAC enhanced the reactor's performance. This comparison is possible because the HRT in the reactors were the same in phases 1, 2 and 4 and the organic loading rate (OLR) applied to each reactor was the same during a particular phase (Table 1). A possible explanation for this difference is the capacity of the PAC in adsorbing soluble compounds such as dye, VFA and aromatic amines inside of the reactor, contributing to the acclimatization of the microorganisms and consequently to a better efficiency of COD removal.

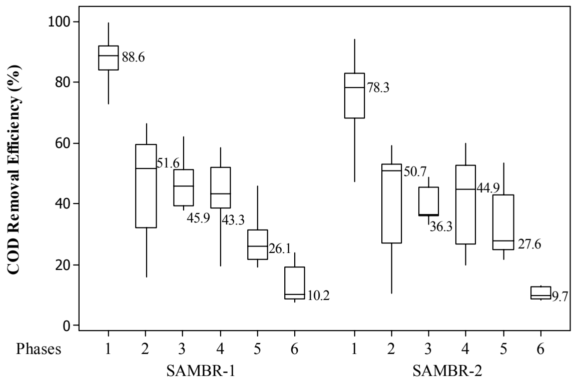

Figure 2: COD removal efficiency in SAMBR-1 (with PAC) and SAMBR-2 (control).

A possible explanation for the reduction in COD removal with the DF decrease is probably related to textile wastewater toxicity and the increase in the toxic load. Vyrides *et al.* (2010) have already shown that changes in some operational parameters, such as the HRT or OLR, can change the salt concentration entering the reactors, thereby causing an osmotic imbalance to the microbial cells. This would contribute to increase cell lysis and the amount of soluble microbial products (especially the more recalcitrant biomass associated products – BAP) in the supernatant, besides reducing the concentration of viable microorganisms in the reactor. This combination of factors seemed to have been detrimental for the observed decrease in the COD removal efficiency. Figure 3 shows that there was a linear relationship between the DF and the COD removal efficiency for both reactors, corroborating the hypothesis of textile wastewater toxicity.

According to Field *et al.* (2000) the anaerobic microorganisms have a low growth rate and are more susceptible to the toxic load. It is well known that wastewater toxicity will be more severe on methanogenic and acetogenic microrganisms, therefore causing an imbalance between the production and consumption of volatile fatty acids (VFA), which could lead to pH reduction and/or thermodynamic constraints in the anaerobic system (Aquino and Chernicharo, 2005). COD due to the VFAs accumulated in SAMBRs 1 and 2 are presented in Figure 4, which shows that, during phases 1 (dilution of 10×), 2 (dilution of 4×) and 4 (no dilution), the COD_{VFA} concentration increased in both reactors. Such an increase can be associated with the stress condition imposed by the increased toxic load, as discussed before.

Other works published by our research group (Baêta *et al*., 2013) have shown that powdered activated carbon (PAC) can be used as adsorbent of toxic byproducts of anaerobic degradation (e.g., aromatic amines) of textile dyes, as well as of some intermediate VFAs, thereby reducing thermodynamic constraints and enhancing the methanogenic and acetogenic activities. A statistical analysis of COD data (Figure 2) in the reactors SAMBR-1 (with PAC) and SAMBR-2 (without PAC) during phases 1, 2 and 4 shows that PAC influenced only phase 1 since there were no statistically significant differences (p-value > 0.05) for the other phases. An explanation for PAC influence only during phase 1 could be the saturation of the adsorption sites as the operation went on. Such a saturation would be enhanced by the decrease in the dilution factor from phases 1 (DF = 10×) to phases 2 (DF= 4×) and 4 (DF = 0×). It is likely that the reduction in the dilution factor caused the increase in the concentration of some compounds that interact strongly with the PAC (e.g., aromatic amines resulting from anaerobic degradation of azo dyes), thereby causing PAC saturation.

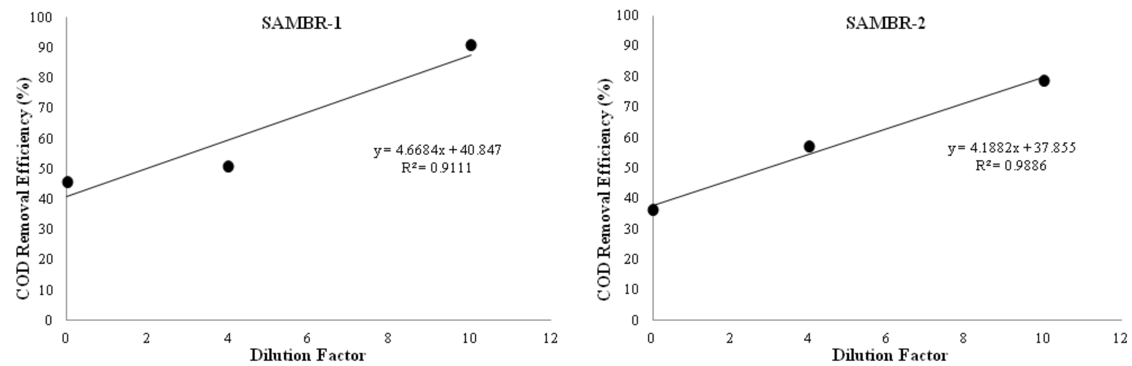

Figure 3: Relationship between COD removal efficiency and dilution factor (DF) in SAMBR-1 and SAMBR-2.

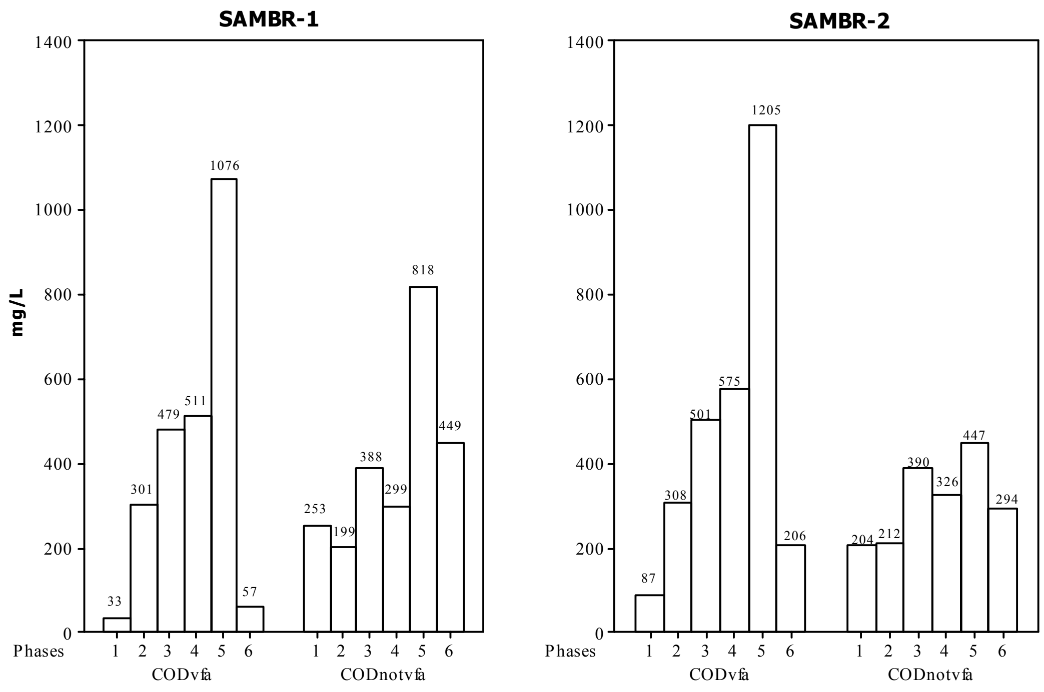

Figure 4: Composition of the COD supernatant in SAMBR-1 (with PAC) and SAMBR-2 (control) during all operational phases.

Nevertheless, the capacity of PAC to adsorb toxic textile wastewater compounds can be observed evaluating the results of COD_{VFA} inside the reactors throughout the operational phases. Figure 4 shows that, in all operational phases, the COD_{VFA} concentration inside SAMBR-1 (with PAC) was lower than SAMBR-2 (without PAC), demonstrating that SAMBR-1 biomass faced less stressful conditions when compared to SAMBR-2 biomass. Under lower stress the acidogenic and methanogenic microrganisms can perform better and contribute to lower VFA accumulation.

The effect of HRT on the COD removal efficiency in both reactors is evaluated by comparing phases 2 and 3. In these phases the dilution factor (DF= 4×) was the same and the HRT was decreased from 24 h (phase 2) to 12 h (phase 3). Figure 2 shows that this caused a reduction in the COD removal efficiency of 5.7% (from 51.6% in phase 2 to 45.9% in phase 3) in the SAMBR-1 and of 14.4% (from 50.7% to 36.3%) in the SAMBR-2. These results are similar to those obtained by other authors such as Peláez -Peláez *et al*. (2011) and a hypothesis to explain such a behavior is the increased toxicity following the flow rate increase. Toxicity would enhance cell lysis and the release of soluble microbial products, as previously demonstrated by Aquino and Stuckey (2004). The stressful condition put upon the anaerobic microorganisms is corroborated by the observed increase in COD_{VFA} inside both SAMBRs. As seen in Figure 4 the COD_{VFA} concentration inside SAMBR-1 increased from 301 to 479 mg/L from phase 2 to phase 3 while for SAMBR-2 the increase was from 308 to 501 mg/L.

By analyzing Figure 2 it is possible to infer that the increase in the organic load in reactors SAMBR 1 and 2 influenced the reactor performance more than the load of salts from the textile wastewater. This is indicated by comparing the COD removal efficiencies in the phases 1 × 2 (different dilution factors) and 2 × 3 (same dilution factor). When phases 1 and 2 are compared, it is possible to see that there was an increase in the OLR (phase 1 = 27.92 $mgCOD.L^{-1}.h^{-1}$ and phase 2 = 58.33 $mgCOD.L^{-1}.h^{-1}$) and in the influent conductivity (phase 1 = 1.57 $mS.cm^{-1}$ and phase 2 = 4.88 $mS.cm^{-1}$). These changes led to a loss of 37% in performance (from phase 1 to 2) in SAMBR-1 and of 28% in SAMBR-2.

When the phases 2 and 3 are compared it can be noted that the OLR increased (phase 2 = 58.33 $mgCOD.L^{-1}.h^{-1}$ and phase 3 = 175.42 $mgCOD.L^{-1}.h^{-1}$), whereas the conductivity remained fairly constant (phase 2 = 4.88 $mS.cm^{-1}$ and phase 3 = 4.23 $mS.cm^{-1}$). In this case there was a loss in performance (from phase 2 to 3) of only 5.7% in SAMBR-1 and of 14.4% in SAMBR-2. These results indicate that reactor performance was much more affected by the conductivity increase (increased load of inorganic matter) than by the OLR increase. In addition, the highest OLRs applied to the reactors (phase 3) are lower than typical specific methanogenic activity (SMA) values of anaerobic sludges (0.2 to 0.25 $kgCOD.kgVSS^{-1}.d^{-1}$), strengthening the hypothesis that inorganic toxicity was more detrimental to the anaerobic consortia than the increased OLR.

During SAMBR operation in the phases 4 (with yeast extract) and 5 (without yeast extract) it was possible to evaluate the effect of a cheap source of riboflavin (a known redox mediator) on color and organic matter removal. In both phases 4 and 5 SAMBR-1 and SAMBR-2 were operated with a HRT of 24 h and fed with raw textile wastewater (without dilution). During phase 4, when both reactors were fed with yeast extract (500 mg/L) the average values of COD removal efficiency were 43.3% for SAMBR-1 and 44.9% for SAMBR-2. When yeast extract was not fed to the reactors (phase 5) the average COD removal efficiency decreased to 26.1% in SAMBR-1 and to 27.6% in SAMBR-2. The differences in the organic matter removal may be explained by the presence of some yeast extract cofactors capable of increasing the growth rate of anaerobic microorganisms. Nevertheless, the COD removal efficiency was too low, even in phase 4, and this is probably due to textile wastewater toxicity, which caused VFA accumulation (Figure 4) and SMP production (Figure 4) in both reactors.

Figure 5 shows the color removal efficiencies in reactors SAMBR-1 and SAMBR-2 during all operational phases, thereby showing the influence of DF and HRT, as well as of PAC and yeast extract addition on textile dyes degradation. During phase 1 the anaerobic reactors were fed with a textile wastewater diluted ten-fold and color removal efficiencies higher than 87% were observed for both SAMBR-1 and SAMBR-2. This indicates that anaerobic microorganisms can efficiently degrade textile dyes as long as the toxic compounds originally found in the textile wastewater or generated during the treatment (e.g., aromatic amines) are present in lower concentration.

When the reactors were fed with less diluted wastewater (phase 2) or with raw textile wastewater (phase 4) a progressive loss in color removal was observed. By decreasing the dilution factor from 10× to 4× and 0× the color removal efficiency decreased from 94% to 74% and 65% in SAMBR-1, and from 87% to 71% and 54% in SAMBR-2, respectively. Such a loss of color removal performance might be explained by an increased concentration of inorganic textile compounds that can interfere in the azo dye anaerobic degradation. Table 1 shows that the values of conductivity of the textile wastewater in phases 1(DF=10x), 2 (DF=4x) and 4(DF=0x) were 1.57, 4.88 and 9.98 (mS/cm), respectively. The higher values of conductivity found in the phases with lower dilution factor may be related the higher concentration of inorganic compounds (e.g., salts) in the textile wastewater.

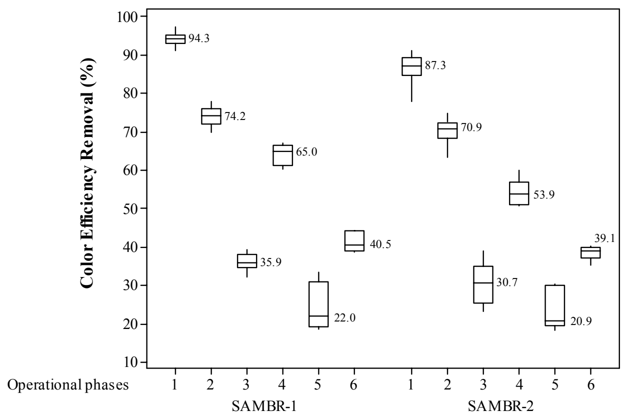

Figure 5: Color removal efficiency in SAMBR-1 and SAMBR-2 during operational phases.

According to Dos Santos (2004) textile effluents contain electron acceptors such as NO_3^-, NO_2^- and SO_4^{-2} which compete with azo dyes for the electrons generated in catabolic anaerobic reactions. As a result, the rate of discoloration is reduced in the presence of such compounds that have reduction potentials higher than that of azo dyes. Another hypothesis for a progressive loss in color removal is the higher amount of dye with complex structure present in the reactors during the reduction of the dilution factor. The color removal in the presence of higher concentration of complex dye may require a higher HRT. This occurs due to the electron transfer limitation caused by steric hindrance of more complex dye molecules.

Figure 5 also shows that the presence of PAC in SAMBR-1 minimized the negative impact caused by reducing the dilution factor. Two hypotheses might explain these results. The first one is related to the PAC capacity of adsorbing dyes and dissolved compounds that interfere with the anaerobic discoloration. The second one is the presence of quinone groups on the PAC surface, which can also act as redox mediators, thereby enhancing color removal, as demonstrated by Baêta *et al*. (2013).

The influence of HRT on color removal efficiency can be evaluated by comparing the phases 2 (HRT= 24 h) and 3 (HRT= 12 h). The reduction of the HRT from 24 h to 12 h caused a huge impact on discoloration performance since color removal decreased from 74% to 36% in SAMBR-1 and from 71% to 31% in SAMBR-2. This behavior may be associated with the increase of the organic and toxic loading rate. The increase of toxic load might have caused a reduction in the metabolic rate of the microorganisms, resulting in a lower production of reduced electron carriers such as NADH and $FADH_2$, regarded as key species to accelerate the discoloration reactions. In addition, the reduction of HRT increases the load of inorganic electron acceptors (nitrate, sulfate) which efficiently compete with the azo dyes for the reducing equivalents produced by anaerobic biomass. The presence of sulfate in the textile wastewater is very likely since sulfuric acid is used to neutralize the pH prior to the biological treatment currently employed. Therefore, the sulfate reducing bacteria (SRB) might play an important role in removing color and COD in the anaerobic SAMBR studied here.

Finally, the use of yeast extract also proved to be efficient in color removal. During phases 4 (with yeast extract) and 5 (without yeast extract) the reactors were fed raw textile wastewater (without dilution) and the presence of yeast extract led to color removal efficiencies 3 times higher in SAMBR-1 and 2.7 higher in SAMBR-2. These results are in accordance with the literature (Baêta *et al*., 2012; Baêta *et al*., 2013), confirming that the kinetics of azo dye (one on the many dye classes found in textile wastewater) degradation is accelerated in the presence of redox mediators such as riboflavin, which have intermediate reduction potential values. It is important to highlight that there is only a handful of studies evaluating the effect of redox mediators on color removal of real textile wastewater.

CONCLUSIONS

- A reduction of the dilution factor from 10× (phase 1) to 4× (phase 2) and 0× (no dilution) led to a reduction in the COD and color removal efficiencies in both anaerobic membrane bioreactors with (SAMBR-1) and without (SAMBR-2) powdered activated carbon (PAC). Such a decrease in reactor performance is likely due to the increased toxic load, which affected the microbial consortium and led to higher volatile fatty acid (VFA) and soluble microbial product (SMP) accumulation.
- PAC adsorbed toxic compounds found in the textile effluent and helped with biomass acclimatization. This fact led to higher COD and color removal

efficiencies in SAMBR-1 when compared to SAMBR-2.

- The dilution factor exhibited a more significant influence on COD removal, while the HRT influenced color removal more in both reactors. In addition, the presence of yeast extract as a source of riboflavin enhanced color removal efficiencies 3-fold in both SAMBRs.

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