

# ANAEROBIC TREATMENT OF CELLULOSE BLEACH PLANT WASTEWATER: CHLORINATED ORGANICS AND GENOTOXICITY REMOVAL

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**Abstract** - This study assessed the removal efficiency of organic matter and how it relates to the decrease of toxic and mutagenic effects when an anaerobic reactor is used to treat the bleaching effluent from two kraft pulp mills. Parameters such as COD (chemical oxygen demand), DOC (dissolved organic carbon), AOX (adsorbable organic halogen), ASL (acid soluble lignin), color, chlorides, total phenols and absorbance values in the UV-VIS spectral region were measured. The acute and chronic toxicity and genetic toxicity assessments were performed with *Daphnia similis*, *Ceriodaphnia sp.* and *Allium cepa L.*, respectively. The removal efficiency of organic matter measured as COD, ranged from 45% to 55%, while AOX removal ranged from 40% to 45%. The acute toxic and chronic effects, as well as the cytotoxic, genotoxic and mutagenic effects, decrease as the biodegradable fraction of the organics is removed. These results, together with the organic load measurement of the effluents of the anaerobic treatment, indicate that these effluents are recalcitrant but not toxic. As expected, color increased when the anaerobic treatment was applied. However, the colored compounds are of microbial origin and do not cause an increase in genotoxic effects. To discharge the wastewater, it is necessary to apply a physico-chemical or aerobic biological post-treatment to the effluents of the anaerobic reactor.

**Keywords:** *Allium cepa L.*; AOX; Genotoxicity; Pulp mill; Toxicity; UV-VIS.

## INTRODUCTION

The manufacture of cellulose pulp is based on a series of processes to degrade and dissolve away the lignin from the wood in order to allow the separation of the wood fibers. The kraft pulping process uses chemicals such as sodium bisulfite and sodium hydroxide. The pulp from the pulping stage has an intense brown color and, for many applications, it needs to be bleached. In this process, unless a totally chlorine free (TCF) bleaching is applied, the effluents have chlorinated compounds derived from the lignin and other wood components. These

compounds can be quantified as AOX (adsorbable organic halogen). AOXs have been listed as contributors to acute, chronic toxicity and mutagenic effects in living organisms (Savant *et al.*, 2006; Rana *et al.*, 2004; Ali and Sreekrishnan, 2001). Authors such as Yan and Allen (1994), Oahn *et al.* (1999) and Savant *et al.* (2006) warned that the compounds with low molecular weight (LMW), smaller than 1000g/mol, pose the greatest toxicological, mutagenic and carcinogenic risk, as they easily penetrate the cell membrane.

However, many of the organochlorine compounds found in these effluents are of high

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molecular weight (>1000g/mol). Although they contribute to toxicity, but to a lesser extent, these are compounds that remain in the environment (Oahn *et al.*, 1999; Springer, 2000). Thus, understanding the contaminants, their origins, degrees of toxicity and the byproducts formed by the applied treatments represent the major problems to be overcome.

The main forms of treatment of effluents from pulp and paper mills have been aerobic processes, with activated sludge as the predominant treatment. However, these treatments are not fully effective for the degradation of organochlorine compounds, proven to be present in these effluents (Leunberger *et al.*, 1985). The pulp and paper mills have therefore invested heavily in the technology of anaerobic treatment, a process favored by the high concentrations of organic matter usually found in industrial effluents (Pokhrel and Virarghavan, 2004).

However, most reactors were installed for the treatment of unsegregated wastewater of the pulp mills, which makes it difficult to adjust the operation to improve the removal of the chlorinated compounds.

A difficulty with biological treatment is the need to achieve high biomass concentration inside the reactor. Particularly the anaerobic treatment requires special attention because the microorganisms actually responsible for the biodegradation of recalcitrant substances have relatively slow growth (Speece, 1996). Therefore, immobilizing the biomass can be the key to a successful treatment of recalcitrant effluents since the low rate of degradation can be compensated by high concentrations of the biomass and prolonged cellular residence times. Therefore, for this work, a horizontal anaerobic immobilized sludge reactor was chosen to treat a kraft pulp bleaching effluent so that the relation between removal of chlorinated compounds and bleach plant effluent genotoxicity could be studied.

## MATERIAL AND METHODS

### Wastewater

The bleaching effluents were obtained from two Brazilian bleached hardwood kraft pulp mills (*Eucalyptus*) using the Elemental Chlorine Free (ECF) process. Mill 1 uses the sequence ZD-EOP-D (chlorine dioxide in the presence of ozone – ZD, followed by alkaline extraction in the presence of oxygen and hydrogen peroxide – EOP, followed by chlorine dioxide – D) and produces approximately 400.000 tons of pulp per year. The second mill, here

termed Mill 2, operates the bleaching sequence Pre-O<sub>2</sub>-DualD-EOP-D (delignification by oxygen – Pre-O<sub>2</sub>, followed by hot chlorine dioxide – DualD, alkaline oxidation extraction with hydrogen peroxide – EOP, followed by chlorine dioxide – D) and produces approximately 630.000 tons of pulp per year, consuming 65.45 m<sup>3</sup>/tons of pulp and 35 m<sup>3</sup>/h of clean water in the bleach plant. The effluents generated in the bleaching stage are of two different types, namely: acidic effluent, after oxidation with chlorine dioxide, and basic effluent, after alkaline extraction. Both effluents are discharged by the plants in 60% and 40% proportions, respectively. These proportions were considered when preparing the mixtures used in this work. Table 1 shows the baseline characteristics of the mixture of effluents from the alkaline and acid stage of both mills, after the pH was adjusted.

**Table 1: Baseline characteristics of the bleaching effluents ( $n=8^{**}$ )**

Characteristic	Unit	Mill 1	Mill 2
		Mean±S.D	Mean±S.D
pH		7.10±0,22	7,3±0,24
Color	C.U	668±64	943±206
COD	mg/L	1148±120	2379±139
CODd	mg/L	859±80	2193±123
DOC	mg/L	335±58	938±51
BOD <sub>5</sub>	mg/L	386±40	1039±105
BOD <sub>5</sub> /COD	----	0.34±0,04	0.45±0.03
Chloride	mg/L	417±93	696±57
AOX	mgCl <sup>1</sup> /L	16±5	22±2
Total phenol	mg/L	208±17	635±49
UV <sub>205</sub> *	cm <sup>-1</sup>	1.41±0.11	1.87±0.12
UV <sub>215</sub> *	cm <sup>-1</sup>	1.09±0.09	1.54±0.09
UV <sub>254</sub> *	cm <sup>-1</sup>	0.40±0.03	0.99±0.07
UV <sub>280</sub> *	cm <sup>-1</sup>	0.31±0.02	0.46±0.02
VIS <sub>346</sub> *	cm <sup>-1</sup>	0.08±0.02	0.09±0.01
VIS <sub>436</sub> *	cm <sup>-1</sup>	0.02±0.009	0.01±0.002
Acute toxicity	TUa	1.46±0.07	15.88±3.40
Chronic toxicity	TUc	----	19.19±1.53

Dilution factor Mill 1 (1:10), Dilution factor Mill 2 (1:20).

TUa = acute toxicological units. TUc = chronic toxicological units

\*\* Except BOD<sub>5</sub> and acute and chronic toxicity, for which,  $n=3$ .

Although the pulp bleaching sequence is elemental chlorine free (ECF) in both mills, there are significant differences in the composition of the effluents. Mill 1, for example, has lower values of all the parameters analyzed, when compared to Mill 2. Note that, although the value of the rate of theoretical aerobic biodegradation in Mill 1 indicates low biodegradability (BOD<sub>5</sub>/COD=0.34±0.04), i.e., the presence of recalcitrant compounds, the effluent from this plant presents lower values of acute toxicity when compared to the effluent from Mill 2 (BOD<sub>5</sub>/COD=0.45±0.03). These differences in the

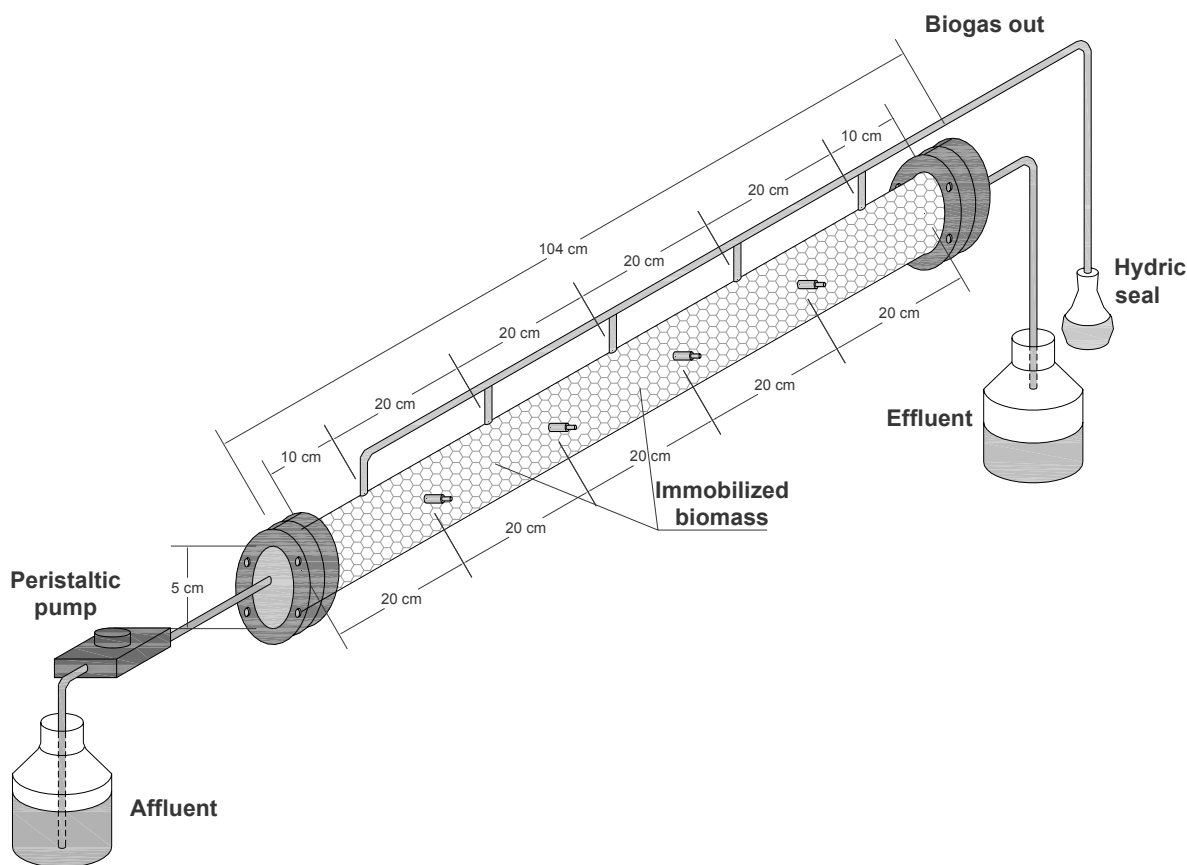
composition of the effluents could be the result of the internal processes of pulp production, particularly at the bleach plant, since, as described, the bleach sequence is not exactly the same in both mills.

### Horizontal Packed-Bed Anaerobic Reactor

The horizontal packed-bed anaerobic reactor (HPBR), developed by Zaiat *et al.* (1994), was chosen for the biological treatment. The principle of this technology is the immobilization of cells in a porous medium that enables the retention of the biomass in a plug-flow reactor. A side benefit of immobilization is an increase of the useful volume of the reactor by reducing the volume for the gas separator. The HPBR reactor was constructed in acrylic, 100 cm in length and 5 cm in diameter, resulting in a L/D ratio equal to 20 and a total volume of 1964 mL. The reactor was placed in a constant temperature chamber set at  $30 \pm 2^\circ\text{C}$ . A 25 g mass of granular sludge from an upflow anaerobic sludge blanket (UASB) reactor treating the

wastewater of a poultry slaughterhouse (Avicola Dakar - São Paulo/SP -Brazil) was immobilized on 0.5 cm polyurethane foam cubes, with an apparent density of  $23 \text{ kg/m}^3$ , following the biomass immobilization methodology proposed by Zaiat *et al.* (1994). The bed porosity ( $\epsilon$ ) was approximately 0.4, which resulted in a net volume of 800 mL for the liquid phase. The reactor was fed with wastewater by means of a peristaltic pump (Provitec/Steck - Dosa mini-4000) with the flow rate adjusted to the desired hydraulic retention time based on the net liquid volume of the reactor. Supplementation of the nutrients was performed with urea and monobasic sodium phosphate, to keep the DQO:N:P ratio equal to 500:5:1, as suggested by Speece (1996). The pH was adjusted with a solution of 0.5 mol/L  $\text{H}_2\text{SO}_4$  or 2 mol/L NaOH when necessary.

The HPBR reactor operated for 418 days, 112 days treating the effluent from Mill 1 and 306 days treating the effluent from Mill 2, with a hydraulic detention time of 25 h. Figure 1 shows a scheme of the HPBR reactor and its components.



**Figure 1:** Scheme of the horizontal packed-bed anaerobic reactor (HPBR).

## Physicochemical Analysis

The determination of COD, BOD<sub>5</sub> and chloride was based on methods described in APHA (2005). To measure CODd (dissolved chemical oxygen demand) and DOC (dissolved organic carbon), the samples were previously filtered through a Millipore cellulose acetate membrane, with 0.45 µm pores. The DOC was determined by using the TOC-5000A meter (Shimadzu). For alkalinity and total volatile acids (TVA), the protocols proposed by Ripley *et al.* (1986) and Dilallo and Albertson (1961) were used. The color was measured according to method H5 proposed by Canadian Pulp and Paper (CPPA, 1993). The AOXs were measured using the W9 method proposed by Scandinavian Pulp and Paper (SCAN, 1989), using the DEXTAR VERTICAL meter (Thermo Electron Corporation).

In order to identify specific groups of compounds based on recent studies reported in the literature (Thomas *et al.*, 1996; Çeçen, 1999; Arslan-Alaton *et al.*, 2002; Langergraber *et al.*, 2004; Chamorro *et al.*, 2005; Lunidquist *et al.*, 2007), a scan was performed of the UV-VIS spectrum between 200 and 346 nm. Çeçen (1999) proposed the use of the following wavelengths and ratios: VIS<sub>346</sub> for lignosulphonic acids; UV<sub>254</sub> for aromatic compounds; UV<sub>280</sub> for lignin derivatives, and the relationships VIS<sub>346</sub>/CODd and UV<sub>280</sub>/CODd.

Authors such as Ghoreishi *et al.* (2007) point out that radiation absorption has been widely used to study the chemistry of lignin and its derivatives and note that currently this technique enables identifying organic compounds by comparison with the spectra of known compounds.

For these measurements, the samples were filtered in a cellulose acetate Millipore membrane with 0.45 µm pores and diluted in acid solution - 3% w/w H<sub>2</sub>SO<sub>4</sub> - to achieve absorbance values of up to 0.9 cm<sup>-1</sup>.

The absorbance values of interest occur at the following wavelengths: 346 nm (lignosulphonic acid), 280 nm (compounds derived from lignin), 215 nm and 205 nm (residual lignin); and 254 nm (chromophoric compounds with conjugated double bonds responsible for the color of these effluents, used indirectly to determine the presence of aromatic carbon). A HACH DR 4000 UV-VIS spectrophotometer and quartz cells of optical path length of 1.00 cm were used.

Based on these values, it was also possible to calculate the following parameters: Specific absorption in the UV (SUVA<sub>254</sub>) proposed by Edzwald (1993), acid-soluble lignin (ASL)

(Goldschmid, 1971; Morais *et al.*, 2005), total phenols, calculated from the absorbance at 215 nm, in the filtered sample (0.45µm Millipore cellulose acetate membrane) and diluted in 0.2 mol/L KH<sub>2</sub>PO<sub>4</sub>, as suggested by Vidal and Diez (2005). Besides these, the relationships VIS<sub>346</sub>/CODd and UV<sub>280</sub>/CODd suggested by Çeçen (1999) were analyzed.

## Biotests

The acute toxicity (EC<sub>50</sub>) expresses the effective concentration that produces adverse effects for 50% of the organisms exposed to the substance or effluent for 48 hours. *Daphnia similis* was chosen as the organism, using the methodology described in ABNT:NBR 12713 (2004). The results were expressed as acute toxicological units (TU<sub>a</sub>=100/EC<sub>50</sub>), calculated according to Hamilton *et al.* (1977).

The chronic toxicity (IC<sub>p25</sub>) tests allow assessment of the more subtle adverse effects on the organisms, for example, reduction in reproduction or growth. *Ceriodaphnia sp.* was chosen as the test organism. The test was performed according to the methodology described in ABNT:NBR 13373 (2005) and the results expressed in chronic toxicological units (TU<sub>c</sub>=100/IC<sub>p25</sub>), calculated according to USEPA (1993).

The test-organism used to evaluate the genotoxic effects were roots of *Allium cepa L.* seeds (2n=16) from the same lot (Baia Periforme variety). This organism has shown good sensitivity for determining the genotoxic effects caused by various substances. The test was performed according to the methodology described by Grant (1982) and Fiskesjo (1994) and adapted by Lemes and Marin-Morales (2008). The end points analyzed were: mutagenic effect (micronuclei), genotoxic effect (chromosomal aberrations, i.e., loss, fragments, bridges, etc.) and the effect on the cell cycle (cytotoxicity) with the mitotic index analysis. These parameters were examined by counting 5000 cells per treatment: positive control (Milli-Q water), negative control (4×10<sup>-4</sup> M methyl methanesulfonate-MMS, CAS:66-27-3, Acros-organics), HPBR feed and effluent. For each test, 500 cells per slide were counted for a total of 10 slides, using an Olympus BX51 microscope coupled to a CoolSnap camera.

The mitotic index, as well as the frequency of chromosome aberrations and micronucleus frequency and losses, were quantified and these results were statistically analyzed using the Kruskal-Wallis method. The effect was considered for levels of confidence  $p < 0.05$  when compared to the negative control.

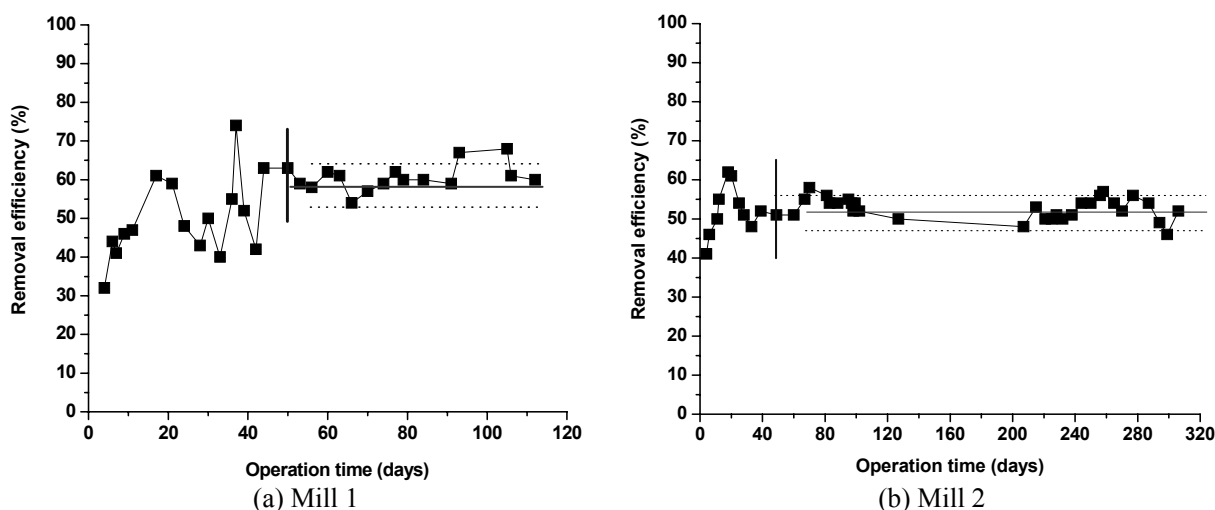
## RESULTS AND DISCUSSION

### Effects on COD, DOC and BOD<sub>5</sub>/COD

Figure 2 shows the results of the temporal variation of the removal efficiency of organic matter measured as COD. It is observed that, for Mill 1 and Mill 2, the process was stable over the operation period, achieved after 40 days (variation range <10% removal efficiency of COD). Considering the measurements performed after adaptation, the average COD removal efficiencies were (55±9)% for Mill 1 and (52±4)% for Mill 2 (Table 2).

Pires and Momenti (2009), operating a HPBR reactor at an hydraulic retention time (HRT) of 12 hours, observed COD removal rates of 50%, while Vidal *et al.* (2007), in anaerobic moving bed reactors, observed that the higher the organic load applied, the lower the COD reduction and the higher the production of color, with COD removal efficiencies of 45% and 59% for organic loads between 0.82 and 0.49 kgCOD/m<sup>3</sup>.day, respectively. Therefore, the removal efficiency of COD attained in this work can be considered to be favorable, since, for the Mill 1 effluent, the applied load of

1.27±0.21 kgCOD/m<sup>3</sup>.day was higher than those reported in the literature. A similar pattern was observed for Mill 2. Although there was an 83% increase of the organic load (from 1.27±0.21 to 2.33±0.19 kgCOD/m<sup>3</sup>.day), the HPBR reactor showed stability in its operation and a decrease of only three percentage points in COD removal efficiency. This decrease in performance is within the experimental variation. The performance of the anaerobic reactor indicates, as observed by Speece (1996), that the already acclimatized and immobilized biomass appears to be a key factor in the increased tolerance to toxic substances. Vidal and Diez (2005) emphasize that the biomass structure is a key factor to consider in the operation of anaerobic biological reactors and that immobilized biomass, or in layers, can significantly increase the methanogenic bacteria tolerance to toxic compounds. Experiments by Fernandez *et al.* (1995 and 2001) show COD removals of 90% upon treating *Eucalyptus* kraft pulp production effluents in an anaerobic USBF (Upflow Sludge Bed Filter). However, despite this result, they observed 50% methanogenic inhibition with 2000 mgCOD/L, a concentration similar to the one found in the current work (Table 2).



**Figure 2:** Efficiency of COD removal by the anaerobic treatment of the effluents from Mill 1 (a) and Mill 2 (b) (the continuous vertical bar indicates the end of the adaptation period).

**Table 2: Average removal efficiency of organic matter by the anaerobic treatment.**

Parameters (mg/L)	Removal efficiencies* (%)	
	Mill 1	Mill 2
COD	55±9	52±4
CODd	52±6	50±6
DOC	57±7	55±8

\* mean ± standard deviation

COD: chemical oxygen demand; CODd: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

With the growing interest in using treatment processes with less energy consumption, the pulp and paper industry is currently studying the use of anaerobic processes for the biological pre-treatment of wastewater.

This work shows that the packed-bed anaerobic horizontal reactor meets that goal. This is a proven fact not only for the removal rates discussed above, but also for the aerobic biodegradation ratio ( $BOD_5/COD$ ), which decreased from  $0.34\pm 0.04$  to  $0.19\pm 0.10$  and from  $0.45\pm 0.02$  to  $0.24\pm 0.05$  between the affluent and the effluent of the HPBR reactor for Mills 1 and 2, respectively. Thus, it was observed that the HPBR effluent is recalcitrant in both cases; therefore, as suggested by Bijan and Mosheni (2005) and Ried *et al.* (2007), it can be treated, for instance, by using advanced oxidation processes (AOP). However, it is important to emphasize that studies on the inhibition of methanogenic activity, the presence of metal ions and compounds, of the sulfur cycle, of wood extractates, resin acids and volatile organic acids should be taken into account in order to better understand the factors that limit the metabolism of the microbial consortium.

Other explanations for the results are found in the works of Rintala and Puhakka (1994), who indicate that the organic matter, measured as COD in anaerobically treated bleaching effluents, is composed of 65% to 75% of chlorolignin polymers, 1% to 25% of methanol, 1% to 5% of carbohydrate and 3% of organic acids. These values are valid if the anaerobic removal treatment is in the range of 28% to 50%. High-molecular weight lignins and tannins are simply not biodegradable in anaerobic environments, possibly causing inhibitory effects on the microorganisms, as well as metabolic disorders (Vidal and Diez, 2005).

### pH Effects, Alkalinity and Total Volatile Acids

The pH of the bleaching effluent (mixed acid-alkaline) was adjusted before feeding the HPBR reactor, maintaining throughout the experiment mean values of  $7.10\pm 0.22$  and  $7.30\pm 0.24$  for Mill 1 and Mill 2, respectively. The optimum pH also depends on the type of microorganism used; for example, the acidogenic bacteria are much less sensitive to pH than the methanogenic ones. Thus, the acidogenic bacteria may prove to be more active than the methanogenic group. This means that the production of acid in a reactor could continue freely, even though the methane production was almost entirely halted. Welander (1988), studying the anaerobic treatment of pulp bleaching effluents, determined that the COD and AOX could have been degraded by acidogenic culture, despite the inhibition of methanogenic bacteria.

During the anaerobic process, the production of alkalinity was observed by the increase in the pH of the effluent of HPBR to  $7.96\pm 0.20$  for Mill 1 and to  $8.60\pm 0.27$  for Mill 2. This was verified by the analysis of partial alkalinity, that corresponded to approximately 80% of the alkalinity due to bicarbonate and total alkalinity values, as suggested by Ripley *et al.* (1986).

The average value of partial alkalinity of the bleach plant effluent from Mill 1 of  $270\pm 114$  mgCaCO<sub>3</sub>/L increased to  $546\pm 73$  mgCaCO<sub>3</sub>/L in the effluent of the HPBR. For Mill 2, the increase was from  $210\pm 159$  mgCaCO<sub>3</sub>/L to  $1287\pm 17$  mgCaCO<sub>3</sub>/L. For the total alkalinity, these values were from  $424\pm 130$  mgCaCO<sub>3</sub>/L to  $689\pm 88$  mgCaCO<sub>3</sub>/L for Mill 1 and from  $548\pm 160$  mgCaCO<sub>3</sub>/L to  $1754\pm 181$  mgCaCO<sub>3</sub>/L for Mill 2. This result indicates that, in both cases, there was the necessary buffering to neutralize the organic acids produced in the acidogenic phase.

Ripley *et al.* (1986) recommend analyzing the intermediate alkalinity/partial alkalinity (IA/PA), suggesting that values between 0.1 and 0.35 indicate stability in the anaerobic digestion and a good performance of the reactors. However, Foresti (1994) emphasizes that these values depend on the origin of the wastewater, and each case should be assessed considering its particularities. For Mill 1, the average IA/AP ratio was  $0.26\pm 0.02$  and for Mill 2 it was  $0.37\pm 0.09$ .

The total volatile acids were reduced from  $118\pm 25$  mgHAc/L to  $45\pm 17$  mgHAc/L for Mill 1 wastewater and from  $569\pm 130$  mgHAc/L to  $301\pm 105$  mgHAc/L for Mill 2 wastewater, again indicating that there was degradation of the biodegradable organic matter and stability of the anaerobic process. It is suggested that this acid reduction could also have been the cause of the pH increase, as already observed.

### Effect on the Absorption Wavelengths in the UV-VIS Spectral Regions

The study of UV absorption for the control and analysis of water quality is a complementary technique for the conventional parameters (COD, BOD<sub>5</sub>, TOC). The absorption of certain wavelengths by the solution studied depends on the chemical nature and concentration of the dissolved fraction, as well as the physical characteristics of the material. Therefore, the shape of the UV spectrum is related mainly to the presence of the colloidal and dissolved fractions, intrinsic parts of the total dissolved solids (Thomas, 1996). From a spectroscopic point of view, a compound absorbs in the UV-VIS region if it contains chromophoric groups, such as residual

lignin or lignin derivatives present in the bleaching effluents.

Thomas (1996) states that, in general, the absorbance decreases as the wavelength increases and the area under the spectrum decreases with water quality improvement. As observed, there was no significant transformation of the organic compounds in the visible and UV region, which contain chromophores. However, as already discussed, there was removal of other organic compounds measured as COD and DOC. The results are similar to the observations reported by Çeçen (1999) and Chamorro *et al.* (2005).

These chromophoric groups indicate the presence of compounds in the residual lignin and low and high molecular weight lignin derivatives. Rintala and Puhakka (1994) point out that, in wood compounds, the high-molecular weight lignin is inherently recalcitrant to anaerobic degradation. However, Vidal *et al.* (2001) point out that the toxicity to methanogenic bacteria is most evident in the low-molecular weight lignin fraction.

Table 3 shows the summary of the average removal efficiency values of organic compounds quantified by the absorbance value in the UV-VIS spectrum region. As observed, the treatment in HPBR was responsible for slight changes in the chromophoric structures of these compounds. This means that the residual COD should be composed mainly of the fraction of high-molecular weight substances present in the wood components after pulp bleaching (lignin, resin acids, chlorophenols, etc.). The removal of 44±10% of the organic compounds, quantified by UV<sub>254</sub>, from the wastewater of Mill 2 is remarkably different from the removal observed for Mill 1. This result indicates that, in Mill 2, the organic matter removed as COD probably has a greater content of a low-molecular

weight fraction of compounds with double bonds that absorb UV<sub>254</sub>. This result could also be ascribed to one of the following factors or a combination of them: the already acclimatized biomass; a co-substrate that favored the natural degradation; a co-substrate from the bleaching effluent that has a greater ability to transform these compounds.

The absorbance values were related to the COD and DOC values, as shown in Table 4, which presents the average of these relationships. The relationships VIS<sub>346</sub>/CODd and UV<sub>280</sub>/CODd showed similar behavior for both mills. This indicates that the lignosulphonic acids, as well as the lignin derivatives, were not mineralized in the same proportion as other organic compounds measured as CODd, confirming that the anaerobic process has factors that limit the degradation of lignin-derived compounds.

Sierra-Alvarez *et al.* (1994) report reductions of between 15% and 20% of lignin-derived compounds, measured as UV<sub>280</sub>, in the UASB reactor and emphasize that the fraction of lignin anaerobically removed and processed corresponds to low-molecular weight lignin derivatives. Vidal *et al.* (2001) confirmed a removal of tannins and lignins of only 10% to 29%, treating bleached pulp effluent in a UASB reactor. Edzwald (1993) and Edzwald and Tobiasson (1999) developed a factor called Specific Ultraviolet Absorption (SUVA) as an operational parameter that indicates the compositional nature of the natural organic matter and the coagulation effectiveness in removing the natural organic matter, the dissolved organic carbon and the trihalomethane precursors. This parameter is defined as the absorbance of the sample at 254 nm divided by the concentration of dissolved organic carbon. It is a parameter that correlates with the aromatic carbon concentration in the humic acid molecule.

**Table 3: Average removal efficiencies of organic compounds measured at different wavelengths in the UV-VIS spectrum, after the anaerobic treatment.**

Wavelength	Removal efficiencies (%)	
	Mill 1*	Mill 2**
UV <sub>205</sub>	23±7	20±7
UV <sub>215</sub>	22±8	19±7
UV <sub>254</sub>	13±6	44±10
UV <sub>280</sub>	17±7	17±9
VIS <sub>346</sub>	13±10	-10±6

\*(DF: 1:10). \*\* (DF:1:20)

**Table 4: Relative and specific average values of absorbance in the UV-VIS spectrum for the anaerobic reactor.**

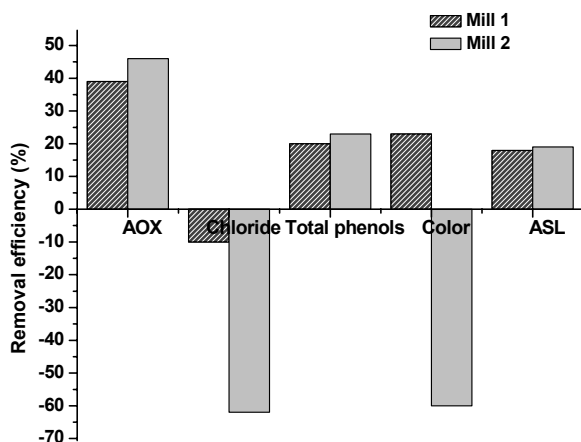
Relations	Unit	Mill 1		Mill 2	
		Influent	Effluent	Influent	Effluent
VIS <sub>346</sub> /CODd	L/mg. m	0.09±0.02	0.17±0.05	0.09±0.01	0.20±0.02
UV <sub>280</sub> /CODd	L/mg. m	0.36±0.04	0.63±0.07	0.42±0.04	0.73±0.07
UV <sub>254</sub> /DOC (SUVA)	L/mg. m	1.28±0.24	2.36±0.50	2.16±0.32	2.72±0.42

The increase in the value of the SUVA relationship in the HPBR effluent for both mills could indicate the presence of microbial degradation by-products, known as SMP (Soluble microbial products). The presence of SMP after anaerobic biological processes has already been reported by Baker and Stuckey (1999). Moreover, it shows the ability of HPBR to mineralize organic compounds different from those that absorb light at 254 nm. As suggested by Edzwald and Tobiason (1999), the SUVA values also indicate that the final bleaching effluent before and after HPBR remains a solution of hydrophobic and hydrophilic compounds of high and low molecular weight.

Although the analysis of the relationship of spectrophotometric values is a relatively new measure for assessing the performance of the treatments, the results of this study suggest that the information obtained provides additional knowledge beyond the conventional global parameter results. It is emphasized that these measurements are quick, economical and fully free of residues that are harmful to the environment.

### Effects on AOX, Chloride, Total Phenols, Color and Acid-Soluble Lignin

Figure 3 shows the average removal efficiencies for AOX, total phenols, color and acid-soluble lignin, and the degree of dechlorination measured indirectly by the chloride determination, as suggested by Çeçen (1999).



**Figure 3:** Average removal efficiency of organochlorine compounds and derivatives of lignin in the anaerobic treatment of the effluents of Mill 1 and Mill 2 (negative values indicate increase instead of removal).

### AOX, Total Phenol and Chloride

As noted, throughout the operational period, the HPBR removed organochlorine compounds measured as AOX with average values of  $39\pm 15\%$  and  $46\pm 6\%$  for Mill 1 and Mill 2, respectively. These values are close to those reported in the literature (between 40% and 60%, Savant *et al.* 2006). Other studies report removal efficiencies of up to 80% when co-substrates such as glucose were added (Rintala and Puhakka 1994). Deshmukh *et al.* (2009), in recent studies treating a pulp bleaching effluent in an anaerobic upflow and fixed bed reactor, showed that the addition of acetate and glucose increased the degradation of AOX to 93%, emphasizing that this value corresponds to the highest AOX removal efficiency thus far reported in the literature for biological processes.

The removal of AOX in the HPBR reactor could be a consequence of the reductive dechlorination phenomenon, since there was dechlorination, quantified as the release of chloride ions, of  $10\pm 16\%$  and  $62\pm 18\%$  for Mill 1 and Mill 2, respectively.

However, other explanations may be considered, among them that there is a natural co-substrate (methanol or ethanol) in the bleaching effluent. Thus, the dechlorination could be a cometabolic process involving the methanogenic bacteria; alternatively, there may be an acclimated microbial consortium, not solely methanogenic, that obtains energy from reductive dechlorination. Savant *et al.* (2006) state that the presence of methanol and ethanol as co-substrates significantly increases the removal of organochlorine compounds, in particular, the chlorophenols. These phenomena are still poorly understood. As suggested by Springer (2000), the fraction of bioaccumulative organochlorines corresponds to only 0.1% of total AOX, composed mainly of phenols, acetones and hydrophilic organochlorine acids and, therefore, easily hydrolysable. Springer also points out that only 0.5% of this fraction is considered to be highly lipophilic and bioaccumulating. As a result, the removal obtained in the anaerobic treatment can be associated with the low-molecular weight fraction of the organochlorine compounds. A removal of total phenols of  $20\pm 4\%$  was observed for Mill 1 and of  $23\pm 7\%$  for Mill 2.

### Acid Soluble Lignin and Color

The color in the effluents from pulp and paper manufacture derives from the presence of residual lignin, of lignin derivatives and polymerized tannins.



It is known that lignin is converted into thio- and alkali-lignin in the kraft process and that lignin and its derivatives are resistant to biological degradation due to the presence of double bonds conjugated with the aromatic rings (Ali and Srekrishan, 2001).

The HPBR showed slight removal of residual lignin and its derivatives, measured as acid-soluble lignin, for both Mill 1 ( $18\pm 8\%$ ) and Mill 2 ( $19\pm 7\%$ ), indicating once again that these types of compounds are resistant to anaerobic degradation. The microbial consortium, while acclimatized, cannot break the conjugated double bonds; thus, there is no significant transformation. Authors such as Ko *et al.* (2008) indicate that anaerobic biodegradation of the high-molecular weight fraction of lignin (recalcitrant) does not occur because the extracellular enzymes necessary for its polymerization use molecular oxygen. However, the same authors point out, based on experimental results, that the high-molecular weight fraction of lignin, under sulfetogenic conditions, can be effectively degraded using cellulose as co-substrate. In the current work, these results were verified by the absorbance decrease at 280 nm of the lignin solution, attributed to the breakdown of the polymer's aromatic structures. Pareek *et al.* (2001) reported similar results, noting that the sulfate-reducing bacteria (SRB) have a higher affinity than the methane-producing bacteria (MPB) to lignocellulose.

However, it is noteworthy that, although the acid-soluble lignin is currently used only in the characterization of the pulping (determination of lignin in the filtrate to calculate the Kappa/ number), it proved adequate for analyzing the residual lignin content in the water samples. However, further studies and correlations using the analytical techniques already established are recommended.

The biological treatments of effluents from pulp and paper mills generally do not result in color removal. On the contrary, on several occasions these treatments do in fact produce color. Moreover, the effluents from elemental chlorine free (ECF) bleaching processes seem to be more susceptible to color formation during the biological treatment, when compared to those using bleaching with elemental chlorine sequences (Milestone *et al.* 2004). However, contrary to expectations, it was observed that in Mill 1 there was a slight removal of chromophoric compounds during the treatment in HPBR, reaching  $23\pm 10\%$  (Figure 3). Kortekaas *et al.* (1998) indicate that the color can be increased by an anaerobic process, as a result of the polymerization of low-molecular weight lignins. Therefore, an explanation of the results could be that the lignin

present in the effluent used in this study is generally of high molecular weight and thus resistant to polymerization and therefore would not cause a color increase. Thus, the microbial consortium is able to reduce other chromophoric groups. This fact can be verified from the reduction of only  $18\pm 8\%$  in the acid-soluble lignin. Vidal *et al.* (2007) observed a clear correlation between the reduction of COD and color: the greater the reduction of COD, the lower the probability of color production. Thus, the results of COD reduction of  $55\pm 9\%$  from the time the process was stable in the HPBR could be considered to be another explanation for the color removal.

In the case of Mill 2, an important color production was observed in the effluent of the HPBR ( $60\pm 18$ ), as shown in Figure 3. However, based on the results of the other parameters (spectrophotometric values, AOX, DOC, COD, ASL), the new compounds that cause this increase in color are not recalcitrant in nature, that is, they do not cause an increase in the residual recalcitrance, as demonstrated in the previous discussion. Therefore, an explanation for this behavior may be that this new color is a result of the generation of soluble microbial products (SMP). This group includes already identified compounds, such as; humic acids, fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, amino acids, extracellular enzymes and structural components of cells. Baker and Stuckey (1999) indicate that SMP production is associated with the increased organic load, which occurred at this stage of the study. However, these results should be verified with additional studies, which can evaluate the influence of the substrate, the organic load applied, the hydraulic detention time, nutrients, effluent toxicity, among other aspects.

### Effect on Acute and Chronic Toxicity

For Mill 1, the acute toxicity was completely reduced in the HPBR reactor. However, one cannot conclude that such removal results from the reduction of organochlorine compounds, measured as AOX, or from the removal of lignin derivatives. Peck and Daley (1994), based on several experimental studies, considered that the organochlorine compounds measured as AOX may not be related to the presence of adverse effects in fish. Instead, these authors suggest that non-chlorinated compounds could be the cause of the harmful effects. However, the qualitative and quantitative aspects of the compound or group of compounds that cause this toxicity are still unknown.

Nevertheless, the acclimated anaerobic microbial consortium proved to be effective in reducing the acute toxic effect and the organic waste that is composed of inherently recalcitrant substances does not present acute toxicity. Although there are no acute toxic effects, it should be noted that there is the possibility that the residual organic load presents chronic toxic effects and mutagenic activity, effects on the organisms that are considered to be sublethal. Pessala *et al.* (2004) note that the analysis of acute toxicity to assess the toxicity of industrial effluents is not enough and suggest that the genotoxicity, chronic toxicity and the effect on the hormonal system must be simultaneously evaluated. Thus, it would be possible to correlate the global parameter limits (AOX, COD, TOC) with more subtle effects on the organisms (Springer, 2000).

The acute toxicity of the bleaching effluent from Mill 2 ( $TU_a = 5.88 \pm 3.40$ ) can probably be attributed, by comparison with the effluent from Mill 1 ( $TU_a = 1.46 \pm 0.07$ ), to the high concentration of low-molecular weight organic compounds, which are more easily hydrolysable. After treatment in the HPBR, although recalcitrant compounds were still present, the effluent showed a 90% decrease in the acute toxic effect and 81% in the chronic toxic effect. However, Sponza (2003) points out that the recalcitrant compounds, although not contributing to the biota toxic effects, because of low degradation and their accumulation potential, could cause negative impacts to the environment.

It is noteworthy that, as discussed for Mill 1, it is not possible to conclude that specific compounds or group of compounds could be the toxic agents. Thus, studies are necessary to isolate and identify these substances or classes of substances, such as applying the TIE method (Toxicity Identification Evaluation). Recent studies by Reyes *et al.* (2009), using the TIE method in an E1 stage effluent of a kraft pulp company, revealed that the compounds responsible

for the *Daphnia magna* acute toxicity were mainly the heavy metals, copper being the main cause of acute toxicity. No studies evaluating the acute and chronic toxicity were found in the literature that analyzes the treatment performance of anaerobic reactors. This highlights the need for studies in this direction, thus complementing the contaminants' degradation assessment and their effects on the receiving bodies.

### Effect on Genetic Toxicity

The genotoxic agents are those that interact with DNA, altering its structure or function. When these changes are set so that they can be transmitted, they are called mutations (Connell, 2005). The assessment of genotoxic effects depends mainly on the test-organisms and the endpoints analyzed (Lemes and Marin-Morales, 2008).

Table 5 shows the results of the mitotic index frequencies, chromosome aberrations and the micronuclei in cells of the meristematic region of roots in *Allium cepa L.* after exposure to the HPBR effluent in Mill 2. The results show that the bleach plant wastewater presents statistically significant differences when compared to the negative control for the three effects studied. Therefore, as quoted in the literature, this wastewater is cytotoxic, genotoxic and mutagenic. Rana *et al.* (2004) also observed that wastewater from bleach plants caused changes in the physiology of the reproductive organs of rodents due to the hormonal function influence. The same authors point out that the effect was more noticeable in males than in females. Therefore, the anaerobic treatment of this kind of wastewater eliminates the cytotoxic and mutagenic effects, either by complete removal of the hazardous compounds or by reducing their concentrations to levels below the harmful limits. Regarding genotoxicity, there is a reduction of this effect, but not a complete elimination.

**Table 5: Results of mitotic index (MI), chromosome aberrations (CA) and micronucleus (MN) in *Allium cepa* meristematic cells after exposure to the effluents of the anaerobic treatment and the negative and positive control treatments.**

Treatment	MI (Cytotoxicity)*	CA (Genotoxicity)*	MN (Mutagenicity)*
Negative control <sup>b</sup>	25.44±2.76	Effect not observed	Effect not observed
Positive control <sup>c</sup>	28.47±4.61	1.20±0.71 <sup>a</sup>	1.61±1.18 <sup>a</sup>
HPBR in	6.04±1.61 <sup>a</sup>	0.99±0.62 <sup>a</sup>	0.73±0.97 <sup>a</sup>
HPBR out	23.69±1.98	0.49±0.44 <sup>a</sup>	0.16±0.21

\*5000 cells analyzed per treatment. Mean±S.D.

<sup>a</sup> Significantly different from negative control ( $p < 0,05$ ), according to the Kruskal-Wallis test.

<sup>b</sup> Milli-Q Water

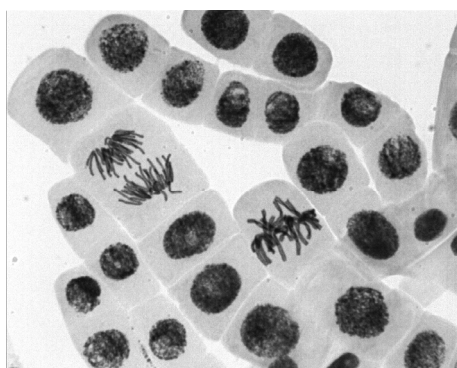
<sup>c</sup> Methyl methanesulfonate MMS 4\* 10<sup>-4</sup>M (CAS:66-27-3, Acros-organics).

The reduction of genetic toxicity, as in the case of acute and chronic toxicity, may be related to the removal of the low-molecular weight fraction of organochlorines, of the residual lignin and lignin derivatives. It is believed that the residual organic matter from the high-molecular weight biological treatment does not cause significant cytotoxic and mutagenic effects, in addition to producing lower genotoxic effects.

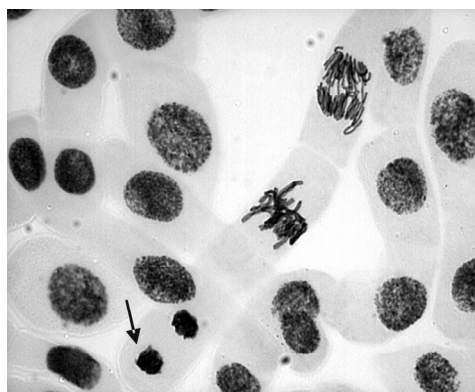
Although several studies are found in the literature on genotoxicity, the vast majority concentrate on specific substance studies. As a

result, studies of complex mixtures, as the case of industrial wastewater and even sewage, need to be considered, since the interaction of these substances in the liquid phase and in the sediments is not known. It is recommended to apply these bioassays before and after treating the effluents.

Figure 4 shows some photographs of the cells of the meristematic region of roots in *Allium cepa* L. exposed to the treatments: negative and positive control, input and output of the HPBR reactor, indicating some of the genetic endpoints observed and quantified.



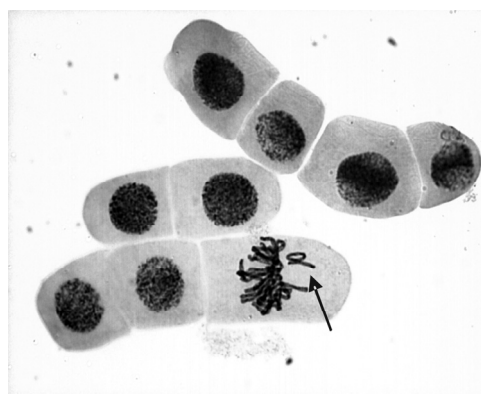
(a) Normal interphase, prophase, anaphase and metaphase.



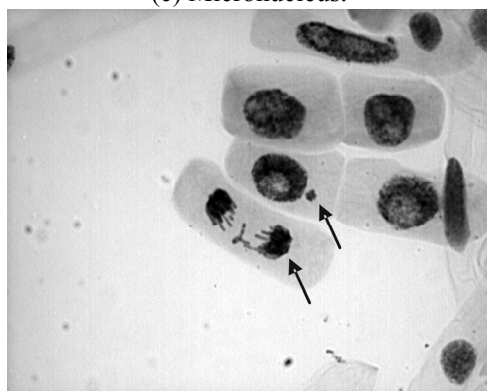
(b) Normal telophase.



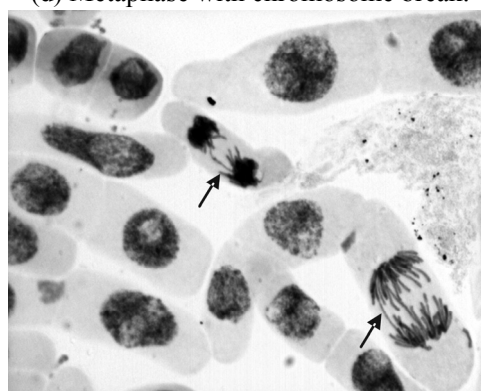
(c) Micronucleus.



(d) Metaphase with chromosome break.



(e) Anaphase with chromosome break



(f) Anaphase with bridge and chromosome loss.

**Figure 4:** Genetic endpoints observed in *A. cepa* exposed to the bleaching plant effluents.

## CONCLUSIONS

The anaerobic treatment of segregated effluents from bleach plant wastewater reduces organochlorine compounds, measured as AOX, by means of reductive dechlorination. Other organic compounds are also removed and, as a consequence, toxic effects decrease substantially.

The decrease of acute and chronic toxic effects and the cytotoxic, genotoxic and mutagenic effects are directly related to the removal of the biodegradable fraction of the organic matter. Data from the literature indicate that the compounds removed belong to the low molecular weight fraction of the wastes.

There is color production, related to the presence of soluble microbial by-products, and despite this increase, the toxic, cytotoxic, mutagenic and genotoxic effects do not increase.

In order to continue removing the residual organic matter, inherently recalcitrant, it is necessary to integrate the anaerobic treatment with advanced oxidation or aerobic post-treatment.

For complex industrial wastewaters, such as the one used in this research, UV/visible spectroscopy together with the ratios of absorbances at different wavelengths provide information that can be related to conventional measurements such as COD and BOD to improve the understanding of the effluent treatment.

Complex wastewaters are subject to unpredictable reactions when submitted to oxidation and the intermediates can be as toxic as the untreated wastewater. Thus, ecotoxicity and genotoxicity tests for different trophic levels are important tools to assure the effectiveness of the treatment.

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## REFERENCES

- Ali, M., Sreekrishnan, T., Aquatic toxicity from pulp and paper mill effluents: A review. *Advances in Environmental Research*, No. 5, p. 175-196 (2001).
- ABNT (Associação Brasileira de Normas Técnicas), Aquatic ecotoxicology – Acute toxicity – Method of assay with *Daphnia* spp. – Cladocera, Crustácea (In Portuguese), ABNT, NBR 12713, Rio de Janeiro, Brazil (2004).
- ABNT (Associação Brasileira de Normas Técnicas), Aquatic ecotoxicology – Chronic toxicity – Method of assay with *Ceriodaphnia* sp. – Cladocera, Crustácea (in Portuguese), ABNT, NBR 13373, Rio de Janeiro, Brazil (2005).
- APHA, Standard Methods for The Examination of Water and Wastewater 21th. Washington, D.C. American Public Health Association/American Water Works Association/Water Environment Federation (2005).
- Arslan-Alaton, I., Balcioglu, I., Bahnemann, D., Advanced oxidation of a reactive dyebath effluent: comparison of O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>/UV-C and TiO<sub>2</sub>/UV-A process. *Water Research*, No. 36, p. 1143-1154 (2002).
- Barker, D., Stuckey, D. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Research*, No. 29, p. 353-364 (1999).
- Bijan, L., Mohseni, M., Integrated ozone and biotreatment of pulp mill effluent and changes in biodegradability and molecular weight distribution of organic compounds. *Water Research*, No. 39, p. 3763-3772 (2005).
- CANADIAN PULP AND PAPER ASSOCIATION, Technical Section Standard Test Methods. *H5*. Montreal: CPPA (1993).
- Çeçen, F., Investigation of substrate degradation and nonbiodegradable portion in several pulp bleaching wastes. *Water Science and Technology*, No. 40 (11-12), p. 305-312 (1999).
- Chamorro, S., Xavier, C., Vidal, G. Behavior of aromatic compounds contained in Kraft mill effluents treated by an aerated lagoon. *Biotechnology Progress*, No. 21, p.1567-1571 (2005).
- Connell, D. W., Basic Concepts of Environmental Chemistry. Boca Raton: 2<sup>nd</sup> Ed., Taylor & Francis (2005).
- Contreras-Lopez, M., Determination of potentially bioaccumulating complex mixtures of organochlorine compounds in wastewater: A review. *Environmental International*, No. 28, p.

- 751-759 (2003).
- Deshmukh, N. S., Lapsiya, K. L., Savant, D. V., Chiplonkar, T. Y., Yeole, P. K., Dhakephalkar, D. R., Ranade, D. R., Upflow anaerobic filter for the degradation of adsorbable organic halides (AOX) from bleach composite wastewater of pulp and paper industry. *Chemosphere*, No. 75, p.1179-1185 (2009).
- Dilallo, R., Albertson, O., Volatile acids by direct titration. *Journal of Water Pollution Control Federation*, No. 33, p. 356-365 (1961)
- Edzwald, J., Coagulation in drinking water treatment: particles, organics and coagulants. *Water Science and Technology*, No. 27, (11), p. 21-35 (1993).
- Edzwald, J., Tobiason, J., Enhanced coagulation: US requirements and broader view. *Water Science and Technology*, No. 40, (9), p. 63-70 (1999).
- Fernandez-Alba, A. R., Hernando, D., Aguera, A., Caceres, J., Malato, S., Toxicity assays: a way for evaluating AOPs efficiency. *Water Research*, No. 29, p. 4255-4262 (2002).
- Fernandez, J., Mendez, R., Lema, J. M., Anaerobic treatment of Eucalyptus fiberboard manufacturing wastewater by hybrid USBF lab scale reactor. *Environmental Technology*, No. 15, p. 667-684 (1995).
- Fernandez, J., Omil, F., Mendez, R., Lema, J. M., Anaerobic treatment of fiberboard manufacturing wastewater in a pilot scale hybrid USBF reactor. *Water Research*, No. 35, p. 4150-4158 (2001).
- Fiskesjo, G., Allium test II: Assessment of a chemical's genotoxic potential by recording aberrations in chromosomes and cell divisions in root tips of *Allium cepa* L. *Environmental Toxicology and Water Quality: An International Journal*, No. 9, p. 235-241 (1994).
- Foresti, E., Fundamentals of anaerobic digestion process (Fundamentos do processo de digestão anaeróbia). In: Maria Vinas, Matilde Soubes, Liliana Borzacconi, Luci Maxi (Org). *Tratamento Anaeróbio*, Montevideo, Universidad de la República. p. 97-110 (1994).
- Ghoreishi, S. M., Haghighi, M. R., Chromophores removal in pulp and paper mill effluent via hydrogenation-biological batch reactors. *Chemical Engineering Journal*, No. 127, p. 59-70 (2007).
- Goldschimid, O., Ultraviolet spectra. In: Lignins: occurrence, formation, structure and reactions. New York: John Wiley Interprice, p. 241-298 (1971).
- Grant, W., Chromosome aberration assays in Allium, a report of the U.S environmental protection agency Gene-Tox program. *Mutation research*, No. 99, p. 273-291 (1982).
- Hamilton, M., Russo, R., Thurston, R., Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology*, No. 11, p. 714-719 (1977).
- Ko, J., Shimizu, Y., Lkeda, K., Kim, S., Park, C., Matsui, S., Biodegradation of high molecular weight lignin under sulphate reducing conditions: lignin degradability and degradation. *Bioresource Technology*, No. 1000, (4), p. 1622-1627 (2009).
- Kortekaas, S., Vidal, G., Yan-Ling, H., Gatzke, L., Field, J., Anaerobic-Aerobic treatment of toxic pulping black liquor with upfront effluent recirculation. *Journal of Fermentation and Bioengineering*, No. 86, (1), p. 97-110 (1998).
- Langergraber, G., Fleischmann, N., Hofstaedter, F., Weingartner, A., Monitoring of a paper mill wastewater treatment plant using UV/VIS spectroscopy. *Water Science and Technology*, No. 49, (1), p. 14 (2004).
- Lemes, D., Marin-Morales, M., Chromosome aberration and micronucleus frequencies in *Allium cepa* cells exposed to petroleum polluted water- a case of study. *Mutation Research*, No. 650, p. 80-86 (2008).
- Leunberger, C., Giger, W., Coney, R., Graydon, J., Molnarkubica, E., Persistent chemicals in pulp mill effluents-occurrence and behavior in an activated sludge treatment plant. *Water Research*, No. 19, (7), p. 885-894 (1985).
- Lunidquist, K., Parkas, J., Paulsson, M., Heitner, C., Reactions of lignin chromophores of the enal and enone types with sulfite. *Bioresources*, No. 2, (3), p. 334-350 (2007).
- Milestone, C. B., Fulthorpe, R. R., Stuthridge, T. R., The formation of colour during biological treatment of pulp and paper wastewater. *Water Science and Technology*, n. 50, (3), p. 87-94 (2004).
- Morais, S., Do Nascimento, E., De Melo, D., Analysis of *Pinnus oocarpa* Wood part I- study of the constituents of the macromolecules and volatile extractives (Análise da madeira de *Pinnus oocarpa* parte I- estudo dos constituintes macromoleculares e extrativos voláteis). *Revista Arvore*, No. 29, (3), p. 461-470 (2005).
- Oanh, N. T., Bengtsson, B. E., Reutergardh, L. B., Hoa, D. T., Bergqvist, P. A., Broman, D., Zebuhr, Y., Persistent organochlorines in the effluents from a chlorine-bleached Kraft integrated pulp and paper mill in Southeast Asia. *Archives of Environmental Contamination and Toxicology*,

- No. 37, p. 303-309 (1999).
- Pareek, S., Azuma, J. I., Matsui, S., Shimizu, Y., Degradation of lignin and lignin molde compound under sulfate reducing conditions. *Water Science and Technology*, n. 44, p. 351-358 (2001).
- Peck, V., Daley, R., Toward a "Greener" pulp and paper industry. *Environmental Science and Technology*, No. 28, (12), 524A-527A (1994).
- Pessala, P., Schultz, E., Nakari, T., Joutti, A., Herve, S., Evaluation of wastewater effluents by small-scale biotest and a fractionation procedure. *Ecotoxicology and Environmental Safety*, No. 59, p. 263-272 (2004).
- Pires, E., Momenti, T., Combination of an anaerobic process with O<sub>3</sub>, UV and O<sub>3</sub>/UV for cellulose pulp bleaching effluent treatment. *Desalination and Water Treatment*, No. 5, 213-222 (2009).
- Pokhrel, D., Viraraghavan, T., Treatment of pulp and paper mill wastewater - a review. *Science of Total Environment*, No. 333, p. 37-58 (2004).
- Rana, T., Gupta, S., Kumar, D., Sharma, S., Rana, M., Rathore, V., Pereira, M., Toxic effects of pulp and paper-mill effluents on male reproductive organs and some systemic parameters in rats. *Environmental Toxicology and Pharmacology*, No. 18, p. 1-7 (2004).
- Reyes, F., Chamorro, S., Yeber, M. C., Vidal, G., Characterization of E1 Kraft mill effluent by toxicity identification evaluation methodology. *Water Air and Soil Pollution*, No. 199, p. 183-190 (2009).
- Ried, A., Mielcke, J., Wieland, A., Schaefer, S., Sievers, M., An overview of the integration of ozone systems in biological treatment. *Water Science and Technology*, No. 55, (12), p. 253-258 (2007).
- Rintala, J., Puhakka, J., Anaerobic treatment in pulp and paper mill waste management. *Bioresource Technology*, No. 47, p. 1-18 (1994).
- Ripley, L., Boyle, W., Converse, J., Improved alkalinity monitoring for anaerobic digestion of high strength wastes. *Journal of Water Pollution Control Federation*, No. 58, p. 406-411 (1986).
- Savant, D., Abdul-Rahman, R., Ranade, D., Anaerobic degradation of sorbable organic halides (AOX) from pulp and paper industry wastewater. *Bioresource Technology*, No. 97, p. 1092-1104 (2006).
- SCAN.W9-Test Method. Estocolmo: Scandinavian Pulp, Paper and Board Testing Committee, (1989).
- Sierra-Alvarez, R., Field, J., Kortekaas, S., Lettinga, G., Overview of the anaerobic toxicity caused by organic forest industry wastewater pollutants. *Water Science and Technology*, No. 29, p. 353-364 (1994).
- Sponza, T., Application of toxicity tests into discharges of the pulp-paper industry in Turkey. *Ecotoxicology and Environmental Safety*, No. 54, p. 74-86 (2003).
- Speece, R., Anaerobic Biotechnology for Industrial Wastewater. Nashville Tennessee: Archae Press (1996).
- Springer, A., Industrial Environmental Control: Pulp and Paper Industry. Atlanta: Tappi Press (2000)
- Thomas, O., Theraulaz, F., Agnel, C., Suryani, S., Advanced UV examination of wastewater. *Environmental Technology*, No. 17, p. 251-261 (1996).
- USEPA, A Linear Interpolation method for Sublethal Toxicity: The Inhibition Concentration (IC<sub>p</sub>) Approach, (Version 2.0). U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN 55804 (1993)
- Vidal, G., Videla, S., Diez, M., Molecular weight distribution of Pinus radiate Kraft mill wastewater treated by anaerobic digestion. *Bioresource Technology*, No. 77, p. 183-191 (2001).
- Vidal, G., Diez, M., Methanogenic toxicity and continuous anaerobic treatment of wood processing effluents. *Journal of Environmental Management*, No. 74, p. 317-325 (2005).
- Vidal, G., Becerra, J., Hernandez, V., Decap, J., Xavier, C., Anaerobic biodegradation of sterols contained in Kraft mill effluents. *Journal of Bioscience and Bioengineering*, No. 104, (6), p. 476-480 (2007).
- Welander, T., Anaerobic process for treatment of CTMP effluent. *Water Science and Technology*, No. 20, (1), p. 143-147 (1988).
- Yan, G., Allen, D., Biosorption of high molecular weight organochlorines in pulp mill effluent. *Water Resources*, No. 28, (9), p.1933-1941 (1994).
- Zaiat, M., Cabral, A., Foresti, E., Horizontal-flow anaerobic immobilized sludge reactor for wastewater treatment: conceived and preliminar performance evaluation (Reator anaeróbio horizontal de leito fixo para tratamento de Águas residuárias: concepção e avaliação preliminar de desempenho). *Revista Brasileira de Engenharia Química*, No. 11, p. 33-42 (1994).