Post-treatment of anaerobic effluent by ozone and ozone/UV of a kraft cellulose pulp mill
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ABSTRACT

Pulp and paper mill effluents represent a challenge when treatment technologies are considered, not only to reduce organic matter, but also to reduce the toxicological effects. Although anaerobic treatment has shown promising results, as well as advantages when compared with an aerobic system, this process alone is not sufficient to reduce recalcitrant compounds. Thus, an advanced oxidation process was applied. This experiment was performed to determine the effect of ozone and ozone/UV treating a horizontal anaerobic immobilized biomass reactor effluent from a kraft cellulose pulp mill for 306 days with an organic volumetric load of 2.33 kgCOD/m³/day. The removal of organic compounds was measured by the following parameters: adsorbable organically bound halogens (AOX), total phenols, chemical oxygen demand (COD), dissolved organic carbon and absorbance values in the UV-visible spectral region. Moreover, ecotoxicity and genotoxicity tests were conducted before and after treatment with ozone and ozone/UV. At an applied ozone dosage of 0.76 mgO₃/mgCOD and an applied UV dosage of 3.427 Wh/m³, the organochlorine compounds measured as AOX reached removal efficiencies of 40%. Although the combination of ozone/UV showed better results in colour (79%) and total phenols (32%) compared with only ozone, the chronic toxicity and the genotoxicity that had already been removed in the anaerobic process were slightly increased. 

Key words | Allium cepa L., bleaching effluents, Ceriodaphnia sp., Daphnia similis, UV-VIS

INTRODUCTION

The pulp and paper industry is a major source of industrial pollution worldwide (Tunay et al. 2010). The characteristics of wastewater generated from the various processes of pulp and paper mills depend upon the types of process and wood materials, technology applied, management practices, internal recirculation of the effluent for recovery, and the amount of water to be used in the particular processes (Pokhrel & Viraraghavan 2004). The bleaching plant is considered to be the main source of pollution in the processing of pulp. In this type of effluent, it is common to find organochlorine compounds derived from lignin and other constituents of wood, which are called organochlorine compounds and quantified as adsorbable organically bound halogens (AOX). However, many of the organochlorine compounds present in these effluents are of high-molecular weight (>1,000 g/mol). While they contribute to toxicity, to a lesser extent, they are persistent compounds in the environment (Oanh et al. 1999). The general belief is that organochlorine compounds are toxic; however, it is important to mention that non-chlorinated compounds such as fatty acids and acid resins, among many other organic compounds, are also toxic (Springer 2000).

The first studies of toxicity using these effluents aimed to analyse only the acute effect, but the United States Environmental Protection Agency considered that this measurement alone was not sufficient to assess the environmental risk of these effluents, and the agency suggested including genotoxic studies. Thus, it would be possible to correlate the limits of both conventional (i.e., chemical oxygen demand (COD), total organic carbon (TOC) and AOX) and non-conventional parameters (i.e., spectrophotometry values) with sub-lethal effects on the organisms.

Anaerobic treatment can be used advantageously as a pre-treatment for pulp mill wastewater to remove not only biodegradable organic matter, but also toxic compounds prior to advanced oxidation processes (AOPs; Chaparro & Pires 2011). Speece (1996) stated that the anaerobic microorganism responsible for the degradation of recalcitrant
compounds has relatively slow kinetic microbial growth. Therefore, the immobilization of biomass can be the key to success for this type of effluent. However, it is known that after biological treatment, both colour and specific organic compounds, which include lignin and some organochlorines, remain in the effluent. For this reason, biological treatment alone may not be sufficient to provide the required effluent quality. Thus, to meet increasingly stringent discharge limits, pulp mills need to adopt technologically advanced treatment systems. Tunay et al. (2010) indicated that oxidation technologies are effective and promising applications for the treatment of pulp and paper industry wastewater. Ozonation, for example, is efficient in removing COD, TOC and colour, as well as increasing the biodegradability in many cases, but it is rather an expensive process. Hence, the most important tasks seem to be the assessment of the conditions when oxidation processes can be applied and how they affect the characteristics of the biologically treated wastewater.

Thus, this research aimed to evaluate organic matter removal and ecotoxicity and genotoxicity after ozone and ozone/UV post-treatment of a kraft pulp mill’s wastewater treated in an anaerobic immobilized biomass reactor. This work could contribute practical knowledge for the treatment of kraft pulp mill wastewater, because the spectrophotometric values and the evaluation of different assays of toxicity and genotoxicity are relatively new measures for assessing the performance of the treatments.

MATERIAL AND METHODS

Wastewater

Raw wastewater was obtained from a kraft pulp mill with an elemental chlorine free (ECF) sequence located in São Paulo state, Brazil. The bleaching sequence used is Pre-O₂-DualD-EOP-D (delignification by oxygen-Pre-O₂ followed by hot chlorine dioxide-DualD, alkaline oxidation extraction with hydrogen peroxyde-EOP, followed by chlorine dioxide-D) and produces approximately 650,000 tons of pulp per year, consuming 65.45 m³/tons of pulp and 35 m³/h of clean water in the bleach plant. Wastewater was collected from the alkaline and acid step of the fully bleached line. The effluents were collected separately and mixed in the laboratory in the ratio of 60:40%. This mixture was the model wastewater for this research.

The wastewater was treated in a horizontal anaerobic immobilized biomass reactor (HAIB) for 306 days with an organic volumetric load of 2.33 kgCOD/m³/day and a hydraulic retention time of 25 h. Further details can be found in Chaparro & Pires (2011). The effluent from this reactor was subjected to ozone and ozone/UV oxidation tests without prior pH adjustment. The pH of the HAIB reactor effluent was close to 8.6.

Ozone and ozone/UV application

Ozone and ozone/UV oxidation experiments were conducted in a bench scale reactor made of a cylindrical tube of bore-silicate glass (internal tube) mounted inside another glass tube (external tube) so that a thermostatic bath enclosed the reactor (external tube diameter = 100 mm, internal tube diameter = 60 mm, height = 54 cm, net volume = 1,200 mL). Ozone was provided by a generator with a production of up to 1.4 gO₃/h and an applied dosage of 0.74 mgO₃/mgCOD. The gas was injected at the bottom of the reactor using a fine bubble plate diffuser. The ozone not consumed in the column was transferred to a separate flask containing potassium iodide solution at 2%. UV-irradiation was performed by means of a low-pressure mercury lamp (15 W, Starlux G15T8) emitting nearly monochromatic light at 254 nm. The lamp was placed axially inside the cylindrical reactor. For every experiment, the treated volume was 800 mL and the irradiation applied dosage was 3.427 Wh/m³. It is worth mentioning that the efficiency of the UV irradiation was conditioned to the notable presence of substances present in the anaerobic effluent that absorb radiation in the wavelength of 254 nm. Each experiment was repeated eight times.

The schematic diagram of the bench-scale oxidation reactor is depicted in Figure 1.

Analytical determinations

To identify the specific group of compounds based on recent studies reported in the literature (Thomas et al. 1996; Ceçen 1999; Arslan-Alaton et al. 2002), a scan was performed of the UV-visible (UV-VIS) spectrum between 200 and 346 nm. The absorbance values of interest occur at the following wavelengths: 346 nm (lignosulphonic acid), 280 nm (compound derived from lignin), 215 and 205 nm (residual lignin); and 254 nm (chromophoric compounds with conjugated double bonds responsible for the colour of these effluents, used indirectly to determine the presence of aromatic carbon). 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.
H2SO4 to achieve absorbance values of up to 0.9 cm⁻¹. A HACH DR-4000 UV-VIS Spectrophotometer and quartz cells of optical path length of 1.00 cm were used. It is worth mentioning that the measures based on the value of the absorbance are a complementary tool to evaluate the performance of the oxidation process. Hence, we measured global parameters as follows: COD, dissolved organic carbon (DOC), AOX, colour and phenols according to the recommendations of APHA (2005). All analyses were performed in duplicate and are reported as average values.

Ecotoxicity and genetic toxicity assays

Acute toxicity with Daphnia similis was performed to determine the wastewater median effective concentration that could immobilize 50% of the organisms within 48 h (EC₅₀) (ABNT 2004). The results are expressed as acute toxicological units (TUₐ, 100/EC₅₀) and were statistically estimated by the trimmed Spearman–Karber method (Hamilton et al. 1977). Subsequently, chronic toxicity with Ceriodaphnia silvestrii was conducted to determine the concentration that could inhibit 25% of the organism’s reproduction (IC₅₀), ABNT (2005) and the results are expressed as chronic toxicological units (TUₐ, 100/IC₅₀). These results were statistically calculated by the linear interpolation method (Norber-King 1993).

To evaluate the genetic toxicity, Allium cepa seeds (variety Baia periforme) were used in each bioassay according to a modified version of Grant’s protocol (1982) and Fiskesjo (1994). The onion seeds were germinated at room temperature (20 ± 5°C) in several Petri dishes, each dish was covered with filter paper and individually wetted with the pulp mill effluent before and after being treated in the HAIB reactor. Control tests were carried out with Milli-Q water (negative control) and methyl methanesulfonate (MMS – Acros organics- CAS 66-27-3) at a concentration of 4 × 10⁻⁴ M (positive control). When the roots reached 2.0 cm in length, approximately 5 days after the beginning of the assay, they were fixed in Carnoy (3:1 v/v) for 48 h. The material was then hydrolysed in HCl 1 N at 60°C for 8 min. The fixed roots were stained with Schiff’s reagent for approximately 2 h in a dark place. To prepare the slides, the meristematic regions were coated with cover slips; to intensify the staining and spread cells, one drop of 2% of acetic carmine was added and later covered with coverslips. The cover slips were removed using liquid nitrogen and the slides were mounted in synthetic resin (Enthelan® – Merck). Genotoxicity was evaluated based on chromosomal aberrations (CA), which was calculated using the expression: CA = (number of cells with CA)/(total number of observed cells) × 100. Cytotoxicity was calculated by the quantification of the mitotic index (MI) using the formula: MI = (number of dividing cells)/(total number of observed cells) × 100 and mutagenic effect was assessed based on the micronucleus (MN) by the formula: MN = (number of cells with MN)/(total number of observed cells) × 100. On each slide, 500 meristem cells of A. cepa were examined, comprising a total of 10 slides (approximately 5,000 cells per treatment). Statistical analysis was performed with the non-parametric Kruskal–Wallis test with significance set at 0.05 using the BioEstat 5.0 software.

RESULTS AND DISCUSSION

It is clear from Table 1 that the effluent from the HAIB reactor requires an extra treatment to reduce the compounds that are recalcitrant to the biological process. As described by Chaparro & Pires (2011), the HAIB reactor meets the goal of reducing the biodegradable fraction present in the cellulose pulp mill.

Effects on COD, DOC, phenols, AOX and color

Regarding COD and DOC, it was observed that the removal efficiency of ozone and ozone/UV treatment was 9% and 12%, respectively. These results agree with the observations of Medeiros et al. (2008) which indicated that the removal of COD and DOC is a consequence of the change in the oxidation state of the carbon present in the organic matter,
meaning there is not a removal of the carbon only a partial oxidation. We verified these results with the value of the biodegradability ratio that increased from 0.18 ± 0.05 to 0.40 ± 0.11. This result was a consequence of the increase in 14% of the biochemical oxygen demand (BOD) and the decrease in 21% of the COD. Moreover, it was calculated using the ratio COD/DOC, and according to Alvaraes et al. (2003) this ratio indicates the mineralization grade. The results showed that for the ozone and ozone/UV the ratio COD/DOC decrease of 2.61–2.54 and 2.53, respectively; suggesting that partial oxidation predominated over the mineralization of the carbon. As seen, there was no difference between the treatment with ozone and ozone/UV.

We observed that with 60 min of reaction time of ozone and ozone/UV the removal of AOX was 31 ± 8% and 46 ± 5%, respectively. The ozone/UV treatment proved to be more effective than the ozone in this case, probably due to the initial conditions of the wastewater that promote the increase of HO• radicals. Mounteer et al. (2005) studied the transformation of organochlorine compounds present in a pulp mill treated with ozone by the ratio AOX/COD. This parameter showed the degree of chlorination, this means the degree of transformation of organochlorine compounds measured as AOX in comparison with other organic compounds, measured as DOC. In particular, the AOX/DOC ratio decreased from 0.027 in the HAIB reactor to 0.020 and 0.016 after ozone and ozone/UV treatment, respectively. This result showed that there was greater transformation of compounds expressed as AOX than other organic compounds. Regarding the total phenols, similar behaviour as with the AOX was observed, since the removal efficiency was 26 ± 2% and 32 ± 1% with ozone and ozone/UV, respectively.

During the biological treatment, as mentioned by Chaparro & Pires (2013), colour was produced. Despite this, it was observed as an important removal of colour both with the application of ozone and ozone/UV, 75 ± 3% and 79 ± 3%, respectively. These results are in agreement with the literature on decolourization by applying ozone.

### Effects on the absorption wavelengths in the UV-VIS spectral region

The absorption of certain wavelengths by the studied solution 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 et al. (1996) states that, in general, the absorbance decreases as the wavelength increases and the area under the spectrum decreases with water quality improvement.

Figure 2 suggests that the ozone and ozone/UV application is transforming the organic fraction of a recalcitrant nature that is present in the HAIB reactor effluent. However, it should be pointed out that there is not much work reported in the literature about the performance of the anaerobic process integrated with the ozone and ozone/UV process, and further results are needed to determine a definitive interpretation. Intermediate by-products should be analysed.

<table>
<thead>
<tr>
<th>Main characteristics of horizontal anaerobic immobilized reactor effluent</th>
<th>COD mg/L</th>
<th>1.125 ± 114</th>
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<tbody>
<tr>
<td>DOC mg/L</td>
<td>428 ± 59</td>
<td></td>
</tr>
<tr>
<td>AOX mg/L</td>
<td>11.70 ± 1</td>
<td></td>
</tr>
<tr>
<td>Phenol mg/L</td>
<td>534 ± 8</td>
<td></td>
</tr>
<tr>
<td>BOD5/COD</td>
<td>0.18 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>UV205 cm⁻¹/C0</td>
<td>1.50 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>UV215 cm⁻¹/C0</td>
<td>1.24 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>UV254 cm⁻¹/C0</td>
<td>0.56 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>UV280 cm⁻¹/C0</td>
<td>0.38 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>VIS346 cm⁻¹/C0</td>
<td>0.10 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Dilution factor: 1:20.
* SD: standard deviation.
* According to Abbas (2011) this value indicates poor biodegradability of industrial wastewater.

![Figure 2](https://iwaponline.com/wst/article-pdf/71/3/382/469610/wst071030382.pdf)
Based on Figure 3(a) and (b), more than 60% of the removal efficiency in the integrated treatment (anaerobic + ozone and ozone/UV) corresponds only to the effect of the ozone and ozone/UV. Except for the compounds that absorb radiation in a wavelength of 346 nm (lignosulphonic acids), which in the HAIB reactor were not removed, instead showed a slight increase in the absorbance value. However, the oxidants removed more than 50% of these compounds.

The combination ozone/UV showed a slight increase of removal efficiency in all UV absorbances. Petala et al. (2008) states that the reduction of UV absorbances could be attributed to the reaction of oxidants with the unsaturated bonds and the aromatic rings of organic compounds, leading to the splitting of bonds and the dissociation of the rings, according to the Criegee mechanisms. Gong et al. (2008) found removal efficiencies of UV254 of 63 and 78% with ozone and ozone/UV, respectively, treating the effluent of a wastewater treatment plant, in the first 30 min. These authors emphasized that ozone reacts in sequence with the aromatic hydrophobic, transphilic and hydrophilic organic fraction whereas the ozone/UV process is less selective and therefore effective for removing all dissolved organic matter.

It should be kept in mind that the oxidation experiments were performed without pH adjustment; therefore, hydroxyl radical reaction mechanisms could be responsible for the oxidation of organics from the anaerobic effluent. In this case, the oxidation activity of radicals might be limited by the presence of scavengers, which causes incomplete degradation of the organic matter of a sample. In particular, the total alkalinity of the anaerobic effluent was 1.754 ± 181 mg CaCO3/L and the partial alkalinity that corresponded to approximately 80% of the bicarbonate alkalinity was 1.287 ± 17 mg CaCO3/L. The presence of bicarbonate ions could have influenced the chain reactions and inhibited ozone and ozone/UV decay to hydroxyl radicals. Furthermore, the color in the anaerobic effluent was rather high, 1,651 ± 144 C.U; hence, the synergetic effect of ozone/UV was not significant if compared to ozonation. This finding is in agreement with Amat et al. (2005), which explains that the performance of ozone/UV treating real wastewater decreases in concentrated samples and recommends that oxidants can be used as a first stage followed by biological treatment.

**Effect on genotoxicity**

Table 2 shows the results for the chromosome aberrations, the MN and the MI frequencies. As described by Chaparro et al. (2010), the bleached plant wastewater presents statistically significant differences when compared with the negative control. Therefore, as cited by several studies and verified in this work, this type of effluent has important genotoxic effects on aquatic ecosystems.

Ozonation led to a decrease on all studied effects. This finding could indicate that both the intermediate by-products and the residual compounds that were not transformed by the ozone do not generate mutagenic, genotoxic and cytotoxic effects. These findings are in accordance with Misik et al. (2011) that explain the impact of ozonation on the genotoxicity of the treated municipal wastewater. In their work, the genotoxic effect was studied at three different trophic levels (i.e., bacteria, plant bioassays and...
mammalian cells). The results showed that in all assays, ozonation reduces genotoxic effects. On the contrary, Petala et al. (2008) studied the effect of ozonation on the mutagenicity (Ames test) of secondary effluents and found that ozone influenced the mutagenicity due to the possible formation of intermediate by-products. Furthermore, these authors conclude that the mutagenicity results were inconsistent, indicating that it is necessary to implement bioassays of different trophic levels.

Regarding the combination of ozone/UV, significant mutagenic effect was observed, but the genotoxic and cytotoxic effects were statistically significant when compared with the negative control. Such a fact can be explained because by-products from the radical reactions when the UV radiation is added can promote genetic disturbances and growth disorders in the cells of superior plants. It is important to note that although the genotoxic and cytotoxic effects decreased after the anaerobic process, in this case the combination of ozone/UV promoted a slight increase of these effects. The authors of the current paper believe that these responses can be related to the presence of low-MW organic compounds in the ozone/UV effluent. This behaviour was verified not only with the increase of BOD5/COD ratio of 50 ± 13% (this increase was the result of an increase of 24 ± 9% in BOD5 and a decrease of 16 ± 5% of COD), but also with the Zahn–Wellens test that showed an increase of 95% of the biodegradability after ozone/UV treatment compared with 87% after ozone application (data in preparation).

Table 2 | Frequency, mean and standard deviation of MI, CA and MN, observed in meristematic cells of A. cepa. Cells were exposed to effluents from the HAIB reactor, ozone and ozone/UV process.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MI</th>
<th>CA</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (Milli-Q water)</td>
<td>25.44 ± 2.76</td>
<td>Not observed</td>
<td>Not observed</td>
</tr>
<tr>
<td>Positive control (MMS)</td>
<td>28.47 ± 4.61</td>
<td>1.20 ± 0.71a</td>
<td>1.61 ± 1.18a</td>
</tr>
<tr>
<td>HAIBin</td>
<td>6.04 ± 1.61a</td>
<td>0.99 ± 0.62a</td>
<td>0.73 ± 0.97a</td>
</tr>
<tr>
<td>HAIBout</td>
<td>23.69 ± 1.98</td>
<td>0.49 ± 0.44a</td>
<td>0.16 ± 0.21</td>
</tr>
<tr>
<td>Ozoneout</td>
<td>25.32 ± 1.78</td>
<td>0.18 ± 0.26</td>
<td>0.12 ± 0.38</td>
</tr>
<tr>
<td>Ozone/UVout</td>
<td>37.18 ± 8.20a</td>
<td>0.42 ± 0.23a</td>
<td>0.22 ± 0.32</td>
</tr>
</tbody>
</table>

*aStatistically different from negative control (p < 0.05). MMS: 4 × 10⁻⁴ M methyl methanesulfonate. 5,000 cells analysed per treatment – Mean ± SD (frequency in 100 cells).

**Effect on acute and chronic toxicity**

The balance between the efficiency of contaminants removal and the toxicological effects of treated effluents is considered an important issue in order to evaluate the effectiveness of the processes applied. This is of particular interest in the case of the application of AOP. For instance, several studies already reported in the literature indicate that after ozone and ozone/UV treatment it is possible that the formation of by-products are more toxic than the parental compounds. (Alvares et al. 2001; Fernandez-Alba et al. 2002; Jamroz et al. 2003).

Figures 4 and 5 show the acute and chronic toxicity measures as toxicological units. As can be seen after the application of ozone and ozone/UV there was not an increase of the acute effects; on the contrary, there was a removal of 91%. But these results are not enough to conclude that the application of AOP does not produce toxic...
by-products as it was observed that after the ozone and ozone/UV the chronic toxicity increased by 48% and 96.5%, respectively. These responses are in concord with the results of genetic toxicity discussed above. Assalin et al. (2007) observed similar behaviour with Pseudokirchneriella subcapitata after ozonization of a kraft pulp mill.

It is worth mentioning that it is not only acute toxicity that must be taken into account to evaluate the toxic effects but chronic and genetic effects must also be studied for pulp and paper mills. The results of this investigation show that it is necessary to study the by-products formed during the reaction and their influence on the toxic effects.

CONCLUSIONS

The effect of ozone and ozone/UV on organic matter removal and different assays to evaluate the toxicity of kraft pulp mill wastewater treated in an anaerobic immobilized biomass reactor was studied in this work. Biological treatment alone is not sufficient to reduce recalcitrant compounds, however, the ozone and ozone/UV post-treatment reached removal efficiencies of organochlorine compounds near 40%. The removal of colour and total phenols was notable; more than 70% of the removal efficiency corresponds only to the effect of the oxidants. Ozone/UV influences the increase in the genotoxicity and cytotoxicity of anaerobic effluent, due to the possible formation of intermediate by-products. Nevertheless, this observation is not conclusive and further studies are still needed.

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