Toxicity reduction and biodegradability enhancement of cork processing wastewaters by ozonation
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ABSTRACT
Biodegradability enhancement and detoxification of cork boiling wastewater (CBW) are required for the successful implementation of biological treatment options. We studied the possibility of achieving these goals through ozonation pre-treatment by experimenting on the effect of ozone dose and pH. The CBW used had a pH of 5.81, a chemical oxygen demand (COD) of 1,865 mg L\(^{-1}\), a biochemical oxygen demand (BOD\(_5\)) of 498 mg L\(^{-1}\) and total phenol (TP) and tannin compounds concentrations of 523 and 399 mg L\(^{-1}\), respectively. The ozone doses ranged from 0.27 to 2.63 for the \(\frac{O_3(\text{applied})}{\text{COD}_0}\) ratios with samples at natural pH and set to 3.33 and 9.96. Ozonation allowed the BOD\(_{20}/\text{COD}\) ratio (biodegradability index) to increase from 0.37 to 0.63 and a toxicity reduction from 3.08 to 1.24 TU (Microtox). The corresponding removals obtained were 15.2–62.0%, 38.4–83.2% and 56.7–92.1% for COD, TP and colour, respectively. The best outcome of ozonation pre-treatment requires \(\frac{O_3(\text{applied})}{\text{COD}_0}\) ratios over 1.5 and an acid pH. The increase of TP removals with ozone dose at acid pH led to biodegradability enhancement and CBW detoxification. However, for similar conditions the highest COD removals were obtained at alkaline pH due to the hydroxyl radicals’ high oxidation ability but lack of selectivity.

Key words | biodegradability, cork wastewater, ozonation, toxicity

INTRODUCTION
The production of cork stoppers used to seal wine bottles is the most valuable product of the cork industry, which uses as raw material the outer bark of the *Quercus suber* L. extracted from cork oak trees older than 20 years, after growth cycles of nine years for about 200 productive years (Pereira 2007). According to Mazzoleni et al. (2005), 15% of the total cork production is used to manufacture cork stoppers, representing two-thirds of cork revenues. Therefore, the economic sustainability of cork production is closely dependent on the public’s preference for stoppers made of cork to the detriment of synthetic materials. This competition can be overcome if cork production and transformation are perceived by consumers as environmentally sustainable activities. However, the first stage of the cork industrial processing is focused on the cleaning, disinfection and moistening of the raw material. For this purpose, the corkwood is immersed in boiling water up to one hour, depending on the contamination of the raw material and type of product; this processing water can be reused from six up to 30 times, which corresponds to a specific water consumption ranging from 0.35 to 0.70 m\(^3\) t\(^{-1}\) (Mazzoleni et al. 2005; Dias-Machado et al. 2006; Mendonça et al. 2007). The resulting effluent presents a dark colour and contains some corkwood extracts such as phenolic acids, vegetable tannins and natural polyphenols. The nature and concentration of these compounds cause the effluent to be considered bio-recalcitrant and toxic (Dias-Machado et al. 2006; He et al. 2007; Mendonça et al. 2007). Summing up, the perception of cork as a ‘green product’ is also dependent on sustainable water use and pollution emission reduction by the cork processing industry.

The efficiency of wastewater treatment options based on biological processes requires wastewater to be biodegradable and non-toxic. In the case of cork boiling wastewaters (CBW), the organic content is high, with the chemical oxygen demand (COD) ranging from 1.6 up to 5.5 g L\(^{-1}\), but the biological oxygen demand for 5 days incubation (BOD\(_5\)) is between 1.1 and 1.8 g L\(^{-1}\), which corresponds to a biodegradability index below the limit of 0.40–0.50 for the BOD\(_5)/\text{COD}\) ratio necessary for considering effluents...
as biodegradable (Gilbert 1987; Acero et al. 2004; Peres et al. 2004). Besides these features, the concentration of total phenols (TP) and tannic compounds, ranging from 287 to 995 mg L\(^{-1}\) and from 250 to 1,700 mg L\(^{-1}\), respectively, is associated with high acute toxicity to the bacterium Vibrio fischeri and the crustacean Daphnia magna, with 4.1 to 12.3 toxic units (TU) and between 3.4 and 11.5 TU, respectively (Mendonça et al. 2004; Peres et al. 2004; González et al. 2006).

Advanced Oxidation Processes have already been tested with CBW to achieve COD removal and biodegradability enhancement. For instance, Benitez et al. (2003) reported COD removals ranging from 12 to 54% with ozonation, and Acero et al. (2004) achieved up to 90% COD reduction for an oxidation stage combining \(\text{O}_3\), \(\text{H}_2\text{O}_2\) and UV radiation. The sequence of Fenton’s reagent and biological treatment, with microbial enriched cultures, resulted in total organic carbon removals above 90% (Dias-Machado et al. 2006) and, in the case of the CBW pre-treatment by ozonation, an increment of COD removal from 13–37% to 67% was achieved by an activated sludge treatment system operating with retention times between 24 and 96 h (Benitez et al. 2003). However, up to date, the effect of pH on CBW ozonation has not yet been studied. The major limitation of the ozonation processes is their operational cost, particularly for close to complete mineralization operations, since the correlation between ozone efficiency and ozone dose is most frequently small (corresponding to a low yield for ozone oxidation). Additionally, the selectivity is influenced by solution pH, radical scavengers’ nature and concentration, causing ozone to be consumed in reactions with non-target compounds (Hoigné & Bader 1976). In fact, to achieve full mineralization of hazardous organic compounds or to accomplish depuration of non-biodegradable effluents, according to environmental standards, by chemical oxidation, the involved costs are high when compared to those of biological treatment options (Oller et al. 2010). However, ozonation is potentially attractive as a pre-treatment stage to enhance biodegradability and reduce toxicity of effluents containing recalcitrant or inhibitory compounds, allowing the depuration process to be completed by biodegradation. Nevertheless, this strategy requires confirmation that the resulting intermediates are assessed for biological degradation and less toxic than the original ones, otherwise incomplete oxidation may increase the toxicity of the wastewater (Lapertot et al. 2007).

Chemical oxidation with ozone is influenced by solution pH; for pH values below 5.5 the hydroxyl radical (OH) formed by ozone self-decomposition is suppressed and molecular ozone (\(\text{O}_3\)) prevails (Hoigné & Bader 1976). Ozone is a very powerful oxidant (\(\text{E}^\circ = +2.07 \text{~V}\)) that can react with numerous organic chemicals, namely with unsaturated organics, in a more selective way than the hydroxyl radicals, which are an extremely strong and non-selective oxidant through chain reactions (\(\text{E}^\circ = +3.06 \text{~V}\)), with potential for oxidizing almost all organic pollutants, but with an effectiveness that can be reduced in wastewaters containing high concentrations of radical scavengers (Masten & Davies 1994; Lapertot et al. 2007).

The objectives of this work were to study the influence of pH and ozone dose in oxidation trials run with CBW samples at natural pH and set close to 3 and 10, to increase biodegradability and reduce toxicity, allowing the depuration process to be completed by biological treatment.

**METHODS**

**Cork processing wastewaters**

The CBW was collected from a cork processing plant located in the Portalegre district (Portugal) and kept frozen at \(-18 \text{~C}\) until use. Prior to any experiment, the wastewater was filtered using 10 and 5 \(\mu\text{m}\) pore size cartridge filters to remove gross suspended solids (SS). In mean terms, the pre-treatment allowed the reduction of the SS concentration by 55% (from 1,305 to 585 mg L\(^{-1}\)).

**Analytical methods**

All the chemicals used were of analytical grade and the analytical determinations were carried out according to Standard Methods (APHA 2005), except for TP and tannin content, which were assessed by methodologies adapted from Folin & Ciocalteu (1927) and Mukkar et al. (1993), respectively. The colour of the samples was measured by the absorbance at 580 nm (dilution 1:2) and the concentration of aromatic compounds at 254 nm (dilution 1:50). The BOD\(_5\) and BOD\(_{20}\) determinations were performed with an Oxitop OC system (WTW, Germany) according to the respirometric method (APHA 2005); this system allows monitoring of the oxygen consumption during the incubation period. The seed used was originally collected from an aeration reactor of an activated sludge system treating domestic wastewater and used after a 6-month preservation period in the laboratory in batch mode operation, using as carbon sources a mixture of glucose and acetate (with a COD of 2 g L\(^{-1}\)), nutrients and micronutrients. The
ecotoxicity was assessed by measuring the inhibition of the V. fischeri exposed to dilutions of wastewater samples before and after oxidation. The bioassays were carried out in a Microtox Model 500 Analyzer with solutions and bacteria provided by Azur Environmental (Berkshire, UK). Samples were tested after pH adjustment to 7.0 and absorbance corrections were performed when sample dilutions were visibly coloured close to the calculated EC50 values. Toxicity data were computed and EC50 values were calculated according to the gamma method, using linear regression analysis of sample dilution as natural logarithm data versus percentage inhibition. All correlation coefficients were >0.90. The test results of these bioassays allowed us to obtain the EC50–5 min and EC50–15 min values, which were the dilution values of the samples that reduced the bacterial light emission by 50% after 5 and 15 minutes exposure, respectively. The dilution values (sample volume/total volume) were converted into TU (corresponding to the inverse of the dilution values). Regarding the oxidation trials, the ozone consumption was calculated by the difference between ozone feed and ozone at the exit of the reactor for discrete intervals of time (10 min) trapped in 200 mL of KI at 2%. The oxidation reaction is terminated by bubbling pure oxygen for at least 15 min before collecting the samples from the closed reaction vessel. Additionally, to ensure complete depletion of residual ozone, we waited at least two hours before starting the bioassays for biodegradability and toxicity assessment.

Experimental set-up

A Fischer Model 502 (Bonn, Germany) ozone generator was employed to produce ozone from dry, pure oxygen. The ozone concentration in the gas phase was in the range of 46–50 mg L⁻¹ and the volumetric flow rate was set to 50 L h⁻¹, corresponding to an average production of 40 mg O₃ min⁻¹. The ozonation trials of the CBW were carried out in a closed vessel of 1 L provided with a mechanical stirrer to promote ozone dissolution in the aqueous medium with applied ozone doses (O₃applied) ranging from 496 to 4,990 mg. The ozonation trials were carried out at 20 ± 1 °C and lasted 10, 20, 40, 60 and 100 min.

RESULTS AND DISCUSSION

The values obtained for the CBW characterization after prefiltration were: pH = 5.81, COD of 1,878 mg L⁻¹, BOD of 498 and 684 mg L⁻¹, after 5 and 20 days’ incubation, respectively. The TP concentration was 523 mg L⁻¹ and the tannic content was 399 mg L⁻¹, the absorbance at 254 nm (dilution 1:50) was 0.562 and the brownish colour measured at 580 nm (dilution 1:2) was 0.554. The main features of this wastewater were the low biodegradability, with a BOD₅/COD ratio of 0.27 and a BOD₉₀/COD ratio of 0.37, and the acute toxicity, measured by the Microtox test, which was 3.08 and 3.46 TU for EC₅₀–5 min and EC₅₀–15 min respectively, suggesting that biological treatment was ineffective. It is worth noting that the BOD of the CBW after 5 days’ incubation did not reach a steady value, possibly because the biological oxidation of the organic compounds, namely low molecular weight and oligomeric polyphenols, such as tannins, phenolic acids, chlorophenols and other organic compounds, proceeds very slowly. Therefore, despite BOD₅ being the parameter usually considered in regulations and the BOD₅/COD ratio used as biodegradability index, we increased the incubation time to 20 days, which proved to be enough to have a constant value for the BOD in all cases. The BOD₅ and BOD₉₀ values show that close to 73% of the biodegradable matter is accessed by microorganisms within 5 days of incubation and the biological oxidation of the remaining fraction is more difficult to accomplish. These values are close to those found in urban wastewater, with a BOD₅/BOD₉₀ ratio of 0.75 but the concentration of organics in the case of the CBW is over the range of values typically found in domestic wastewaters, with BOD₅ values ranging from 100 to 300 mg L⁻¹ (Metcalf & Eddy 1993). Therefore, CBW pre-treatment is required to increase the viability of biological treatment systems.

The alteration of the samples’ pH is required for several physicochemical treatment processes, namely with Fenton’s reagent (Dias-Machado et al. 2006), coagulation/flocculation (Peres et al. 2004) and solar photocatalytic process (Vilar et al. 2008) and was tested by us for ozonation experiments with CBW for O₃(applied)/COD₀ ratios ranging from 0.27 to 2.52, 0.27 to 2.63 and 0.28 to 2.26, for acid (pH 3.33), natural (pH 5.81) and alkaline pH (pH 9.96), respectively. The alkaline pH tends to allow hydroxyl radicals to prevail to the detriment of molecular ozone during the oxidation reactions. However, setting the pH close to 10 only guarantees a pH over 5.5 for oxidation reactions up to 20 min; for longer reaction times the prevailing oxidant will be molecular ozone. The pH decay with ozonation is a well-known consequence of the conversion of pollutants into organic acid of lower molecular weight. The yield for the supplied ozone, corresponding to the ratio between consumed ozone and supplied ozone, was always higher for
alkaline pH, ranging from 40.3 to 90.8%, and was between 42 and 85.3%, and 32.6 and 79.4% for natural and acid pH, respectively. The reduction of COD at alkaline pH was also above the values achieved for the other pH values (Figure 1(a)). For the highest ozone doses (corresponding to 4,235, 4,788 and 4,990 mg of O₃ for alkaline, acid and natural pH, respectively), the COD reductions were 53.4%, 58.8 and 62% for acid, natural and alkaline pH, respectively. It is worth noting that the ozone yield decays with reaction time and was below 50% after 60 min reaction (ozone doses ranging from 1,324 to 1,782 mg), which limits the practicability of ozonation and points to the need to drive the oxidation for biodegradability improvement and detoxification to the detriment of mineralization. Ozone doses corresponding to O₃/applied/COCOD₀ ratios ranging from 1.50 to 1.72 (60 min reaction) were sufficient to increase the BOD₂₀/COD ratio up to 0.50 for samples at acid and natural pH and over 0.40 for the alkaline pH values (Figure 1(b)), allowing biodegradation.

As the ozonation time increases from 10 to 100 min (corresponding to O₃/applied/COCOD₀ ratios ranging from 0.27 to 2.63), the remaining substrates are increasingly recalcitrant to oxidation and the stoichiometric ratios (corresponding to the ratio between COD removal and the amount of consumed ozone) exhibit a significant decay from 0.72 to 0.65, from 0.70 to 0.52 and from 0.96 to 0.56, for acid, natural and alkaline pH, respectively, showing another important economic constraint. For these ozone dose ranges, the colour removals were 56.7–90.8%, 58.7–92.1% and 66.0–83.2% for oxidation trials at natural, alkaline and acid pH, respectively. However, the oxidized effluents were slightly brownish coloured in all cases. Similar reductions of the absorbance at 254 nm, resulting from the concentration of aromatic compounds, were achieved and corresponded to the direct or indirect attack of molecular ozone or free radicals produced by ozone decomposition, respectively, on compounds having carbon-carbon double bonds (Masten & Davies 1994).

The biodegradability increase was more dependent on ozone selectivity than on COD removal, since smaller COD reductions at acid pH corresponded to a higher BOD₂₀/COD ratio increase (Figure 1). Therefore, the increase of ozone doses supplied at alkaline pH may result in the oxidation of biodegradable pollutants instead of the toxic and recalcitrant ones. Interestingly, the advantage of molecular ozone for CBW biodegradation can be correlated with the degradation of phenolic compounds (Figure 2(a)); which is very fast at pH 3.33, since with an O₃/applied/COCOD₀ ratio of 1.10 the reduction obtained was already 76.9 and only increases up to 83.2% with an ozone dose.

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**Figure 1** | Influence of pH and ozone doses on the reduction of COD (a) and biodegradability enhancement (b).

**Figure 2** | Variation of the biodegradability (BOD₂₀/COD ratio) (a) and toxicity (b) as a function of pH and total phenols removal.
rise to 2.52. TP degradation ranged from 38.4 to 74.6% at alkaline pH and corresponded to the less favourable conditions, with the natural pH allowing intermediate removals. These results are possibly the consequence of the lower selectivity of the hydroxyl radicals when compared to molecular ozone for the oxidation of phenolic compounds (Hoigné & Bader 1985).

The other goal of ozonation pre-treatment, the CBW detoxification, was also accomplished at acid and natural pH. But, in these cases, the correlation between TP removal and toxicity was not so clear, namely for alkaline pH (Figure 2(b)). However, these results may be a consequence of the high sensitivity of V. fischeri bacteria to the large variety of compounds resulting from the lack of selectivity of hydroxyl radical reactions, which are reacting with other pollutants rather than phenolic compounds. Therefore, the best results for ozonation pre-treatment are obtained when the oxidation is based on the selectivity of molecular ozone for phenolic compounds. It is also worth noting that the concentration of tannin compounds closely follows the variation reported for phenolic compounds.

CONCLUSIONS

The organic load on the CBW sample was high, with a COD of 1,878 mg L\(^{-1}\); but having a low biodegradability of 0.27 and 0.37 for the BOD\(_5\)/COD and BOD\(_{20}\)/COD ratios, respectively. Additionally, only 73% of the biodegradable matter is easily accessed by microorganisms and the Microtox bioassay revealed a high acute toxicity of 3.08 TU for an exposure time of 5 min. These features prevent the successful application of biological treatment. Therefore, we studied the possibility of achieving CBW biodegradability enhancement and detoxification by ozonation pre-treatment, allowing depuration to be completed by biodegradation. The ozone doses experienced ranged between 0.27 and 2.63 for the O\(_3\)(applied)/COD\(_0\) ratios with samples at natural pH (5.81), set to acid (3.33) and alkaline (9.96). For similar ozone doses, the highest COD removals were obtained at alkaline pH, with 25–62%; and with the TP concentration reduction the highest percentages were obtained at acid pH and ranged from 66.0 to 83.2%. However, in order to increase biodegradability it is preferable to have TP concentration reduction rather than COD removal, since for higher TP removals we obtained increments of the BOD\(_{20}\)/COD ratio up to 0.63 at acid pH. Therefore, it is preferable to set reaction conditions that allow the presence of molecular ozone to the detriment of the more reactive but less selective hydroxyl radicals, i.e. pH values below 5.5 (acid). The correlation between TP concentration and sample toxicity was also established but was less evident.

The major limitation of the ozonation pre-treatment option results from the significant ozone yield decay with reaction time, which was below 50% after 60 min reaction (corresponding to ozone doses between 0.71 and 0.95 for the O\(_3\)(applied)/COD\(_0\) ratio). However, these ozone doses were sufficient to increase the CBW BOD\(_{20}\)/COD ratio over 0.40 for alkaline pH and over 0.50 for acid to natural pH values.

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