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Effects of gamma radiation on cork wastewater: Antioxidant activity and toxicity

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HIGHLIGHTS

- Real cork boiling wastewater as case study.
- Gamma radiation was studied as advanced oxidation process.
- Evaluation of the antioxidant activity of cork wastewater after gamma radiation.
- Toxicity assessment performed to study the effects of gamma radiation.

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ABSTRACT

A comprehensive assessment of the toxicity and antioxidant activity of cork boiling wastewater and the effects of gamma radiation on these parameters was performed. Antioxidant activity was evaluated using different methodologies as DPPH radical scavenging activity, reducing power and inhibition of β-carotene bleaching. The results have shown that gamma radiation can induce an increase on the antioxidant activity of cork boiling wastewater. Toxicity tests were performed to access the potential added value of the irradiated wastewaters and/or minimization of the impact for discharge in the environment. Two different methods for toxicity evaluation were followed, bacterial growth inhibition test and cytotoxicity assay, in order to predict the behavior of different cells (prokaryotic and eukaryotic) in the presence of cork wastewater. Non-treated cork boiling wastewater seemed to be non-toxic for prokaryotic cells (*Pseudomonas fluorescens* and *Bacillus subtilis*) but toxic for eukaryotic cells (A549 human cells and RAW264.7 mouse cells). The gamma radiation treatment at doses of 100 kGy appeared to increase the toxicity of cork compounds for all tested cells, which could be related to a toxic effect of radiolytic products of cork compounds in the wastewaters.

1. Introduction

Cork industry is one of the most important industries in Portugal. Cork is the bark of the oak tree which is periodically extracted in 9–12 years in order to produce cork with desirable properties for industrial processing. This raw material is used in a large variety of products ranging from engine gaskets to wine stoppers (Mazzoleni et al., 2005).

Cork processing includes the cork planks immersion in boiling water during 1 h to improve their chemical characteristics, such as elasticity and homogeneity and to make it flat. Normally, the same water is used for several boiling cycles which concentrates it in persistent compounds. The resulting effluent is an aqueous and complex dark liquor with high concentration of cork extracts such

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as phenolic acids, tannins (Minhalma and Pinho, 2001) and 2,4,6-trichloroanisol which are difficult to degrade by conventional treatments. The wastewater produced during this stage represents the main source of wastes (approximately 1500 L per ton of cork). It has low pH and contains high contents of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD). The nature and the concentration of phenolic compounds make this water a toxic and recalcitrant effluent (Dias-Machado et al., 2006). Based on this fact, this effluent might be a risk for the ecosystem, requiring previous treatment before discharge.

In the literature, studies have been published in the last years about the toxicity of some components of cork wastewater like phenolic compounds mixtures using microcalorimetric method (Chen et al., 2010) and also using *Vibrio fischeri*, *Daphnia magna* and *Lemna minor* as test organisms to evaluate acute and chronic aquatic toxicity (Gomes et al., 2013; Mendonça et al., 2007). Although cork wastewater was classified as ecotoxic for some tested species, the biological effect of real cork wastewater is still unclear, since it is considered that the evaluation of toxicity should be done with organisms that have the ability to persist in a wide range of environments, including soils and surface of plants.

Taking into account this problematic and the inefficiency of the conventional treatments to eliminate the recalcitrant compounds, the use of advanced oxidation processes (AOPs) has become widespread in the world. Several processes for cork boiling wastewater treatment have been reported, such as ozonation (Acero and Benitez, 2004; Gomes et al., 2013; Torres-Socías et al., 2013), Fenton's reagent (Dias-Machado et al., 2006; Guedes et al., 2003) and membrane separation (Benítez et al., 2008; Oliveira et al., 2009; Teixeira et al., 2009). Silva et al. (2004) used the combination of H_2O_2, TiO_2, Fe^{2+} and UV radiation to treat cork cooking wastewater being the photo-Fenton process the more efficient. More recently, Torres-Socías et al. (2013) studied the remediation of cork boiling wastewater using solar photo-Fenton and ozone (alone or in combination with H_2O_2) with different flocculants as physico-chemical pre-treatment. All of these processes proved to be effective for COD removal and biodegradability enhancement, although the use of chemicals increase the pollutant load of wastewaters and can form other species that could interfere on the degradation of the desired compounds (Moon et al., 2011). In a recent study, the efficiencies and equivalent costs of advanced oxidation processes (AOPs) such as ionizing radiation radiolysis, photocatalysis, photolysis and ozonolysis were investigated for the treatment of a simulated textile dye wastewater (Guin et al., 2014). Among these AOPs, the most effective in the mineralization of the effluent at the lowest cost was the ionizing radiation treatment, with a total cost/m^3 of wastewater of 175€. The ionizing radiation is being considered an emergent technology for wastewater treatments (Guin et al., 2014). For example, the use of gamma radiation has been studied as a promising technology with the capacity to reduce the impact of chemical and biological pollution of effluents in the environment (Cabo Verde et al., 2015; Melo et al., 2008). In addition, gamma radiation has been also used to reduce the toxicity and mutagenicity of other industrial effluents. Namely, Jo et al. (2006) observed that gamma radiation (absorbed dose of 20 kGy) decreased the toxicity of the textile raw wastewater. Iqbal et al. (2015) studied the effect of different absorbed doses of gamma radiation on the cytotoxicity and mutagenicity of an effluent of a textile industry. In fact, these authors observed that gamma radiation is a feasible technology for detoxification of pollutants and for the degradation of toxic agents. However, Park et al. (2008) suggested that the use of gamma rays seems to be not effective in the reduction of toxicity of wastewater from a rubber products factory and other toxicants than the destroyed organic compounds could formed. In practice, radiation based pilot sludge treatment plants have been established in New Mexico, USA (gamma radiation); Weldel, Germany (e-beam); Verginia Key, USA (e-beam); Takasaki, Japan (e-beam); Sao Paulo, Brazil (e-beam); Tucuman, Argentina (Gamma); Daejeon, Korea (e-beam) (Cooper et al., 1998; Guin et al., 2014). Furthermore, radiation based commercial sludge treatment plants were also established in Vadodara, India and Munich, Germany (Cooper et al., 1998; Guin et al., 2014). A pilot plant for treating 1000 m3/day of dyeing wastewater with e-beam has been constructed and operated since 1998 in Daegu, Korea in conjunction with the biological treatment facility (Han et al., 2012; Maruthi et al., 2011).

Concerning the cork industry, there are few feasibility studies about gamma radiation in the cork boiling wastewater treatment (Lima et al., 2016; Madureira et al., 2013). The authors observed that this technology could bring added-value to this effluent by increasing the antioxidant activity, while decreasing the organic matter (Madureira et al., 2013). The phenolic compounds present in cork waters are known for their high antioxidant activity (Benitez et al., 2003) and their recovery could represent a potential way to valorise the wastewater from cork industry. On the other hand, our previous studies also indicated a negative effect of radiolytic by-products of cork wastewater model solution on the growth of a microbial community, conducting to the hypothesis that irradiated sample toxicity could increase due to the generated intermediates (Lima et al., 2016). These studies raised some issues to investigate, and further studies are needed to elucidate the effects of gamma radiation on the treatment and potential valorization of cork wastewater.

The aim of this work was to study the antioxidant activity and toxicity of cork cooking water and the influence of gamma radiation treatment on these parameters. Two different methods for toxicity evaluation were applied in order to predict the behavior of different cells (procaryotic and eukaryotic). *Pseudomonas fluorescens* and *Bacillus subtilis* were used in the growth inhibition test as previously reported by Paran et al. (1990). These microorganisms were different from the ones usually used to assess the toxicology of wastewaters and especially from cork industry (Mendonça et al., 2007). On the other hand, two different eukaryotic mammalian cell lines, human and mouse, were used to compare the potential cytotoxicity effects of cork wastewater compounds in different living beings (Ma et al., 2014).

2. Materials and methods

2.1. Reagents, cells and bacterial strains

The cork boiling wastewater samples (5 L) were collected from cork planks immersion boiling tanks at *AMORIM Industrial Solutions*, a production and transformation cork industry located in Coruche, Portugal.

For chemical characterization: gallic acid and Folin-Ciocalteau reagent were obtained from Sigma (St. Louis, MO, USA). Sodium carbonate and sulphuric acid (95–97%) were from Merck (Kenilworth, NJ, USA). Ethanol PA was acquired from Panreac Química SA (Barcelona, Spain) and potassium hydrogen phthalate from Fisher Scientific (Waltham, MA, USA). Ferrous ammonium sulfate (FAS) was acquired from Carlo Erba (Val de Reuil, France) and potassium dichromate from JMGS (Odivelas, Portugal). Mercury sulfate and silver sulfate were purchased from VWR (Radnor, PA, USA).

For antioxidant activiy analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources.