Ecotoxicology and Environmental Safety 115 (2015) 234–242

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Biological treatment with fungi of olive mill wastewater pre-treated by photocatalytic oxidation with nanomaterials

V. Nogueira [a,b,*], I. Lopes [a,b], A.C. Freitas [b,c,d], T.A.P. Rocha-Santos [b,d], F. Gonçalves [a,b], A.C. Duarte [b,d], R. Pereira [e,f]

[a] Department of Biology, University of Aveiro, Campus Universitário de Santiago, P-3810-193 Aveiro, Portugal
[b] CESAM (Centre for Environmental and Marine Studies), University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[c] ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, Lordosa, 3515-776 Viseu, Portugal
[d] Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, P-3810-193 Aveiro, Portugal
[e] Department of Biology, Faculty of Science, University of Porto, Rua do Campo Alegre 4169-007 Porto, Portugal
[f] Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P-4050-123 Porto, Portugal

ARTICLE INFO

Article history:
Received 16 November 2014
Received in revised form
16 February 2015
Accepted 17 February 2015
Available online 24 February 2015

Keywords:
Olive oil mill wastewater
Biological treatment
P. chrysosporium and *P. sajor caju*
Photocatalytic oxidation
Nano-TiO_2 and nano-Fe_2O_3

ABSTRACT

Olive mill wastewater (OMW) still is a major environmental problem due to its high chemical oxygen demand (COD) and total phenolic content (TPC), contributing for the high toxicity and recalcitrant nature. Several attempts have been made for developing more efficient treatment processes, but no chemical or biological approaches were found to be totally effective, especially in terms of toxicity reduction. In this context, the main purpose of this study was to investigate the treatability of OMW by the combination of photocatalytic oxidation, using two nanomaterials as catalysts (TiO_2 and Fe_2O_3), with biological degradation by fungi (*Pleurotus sajor caju* and *Phanerochaete chrysosporium*). Photocatalytic oxidation was carried out using different systems, nano-TiO_2/UV, nano-Fe_2O_3/UV, nano-TiO_2/H_2O_2/UV and nano-Fe_2O_3/H_2O_2/UV. The effectiveness of the treatment was assessed through color (465 nm), aromatics (270 nm), COD and TPC reductions, as well as by the decrease in toxicity using the bacterium *Vibrio fischeri*. The chemical treatment with the system nano-TiO_2/H_2O_2/UV promoted 43%, 14%, 38% and 31% reductions in color, aromatics content, COD and TPC, respectively. However no toxicity reduction was observed. The combination with a biological treatment increased the reduction of COD and TPC as well as a reduction in toxicity. The treatment with *P. chrysosporium* promoted the highest reduction in toxicity, but *P. sajor caju* was responsible for the best reduction in COD and TPC. However, the biological treatment was more effective when no hydrogen peroxide was used in the pre-treatment.

1. Introduction

Olive oil production is an important economic activity in the Mediterranean countries. However, this agro-food industry produces a meaningful negative externality, namely a high volume of a wastewater particularly difficult to threat, which is responsible by serious environmental problems. Olive oil mill wastewater (OMW) is characterized by a foul-smelling, dark color, acidic pH and high organic content; being mainly composed by sugars, tannins, pectins, polyphenols, polyalcohols and lipids (Dermeche et al., 2013). The high levels of chemical and biological oxygen demand (COD and BOD levels) and the high content in recalcitrant phenolic compounds are responsible by ecotoxic, phytotoxic and microbial growth inhibition effects of the OMW (Asfi et al., 2012; Justino et al., 2009). However, although the well known toxicity of this effluent it continues to be discharged in freshwater ecosystems or used for ferti-irrigation without proper treatment (Justino et al., 2012; Pavlidou et al., 2014). This procedure is especially common in some Mediterranean countries, like Portugal and Italy, where legislation (Law no. 626/2000 and Law no. 574/1996) for the application of OMW in agricultural soil is already available. According to a recent report of Prosodol project (Prosodol, 2012) in Spain the most widely used extraction process is the 2-phase system with the production of new concentrated waste called "alpejuro" or wet pomace, however in other countries like Italy, Greece and Portugal the most common processes are the 3-phase and the traditional pressing systems with the production of huge quantities of OMW. It is then of crucial importance to find effective treatments to threat OMW before its safe disposal in the environment.

Thereby, several methods were already tested, including

* Corresponding author at: CESAM & Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
Fax: +351 234 372 587.
E-mail address: v.ines@ua.pt (V. Nogueira).

http://dx.doi.org/10.1016/j.ecoenv.2015.02.028
0147-6513/

physical, chemical and biological processes, aimed in promoting the degradation of the complex organic load of this effluent (Karakaya et al., 2012; Kilic and Solmaz, 2013; McNamara et al., 2008; Paraskeva and Diamadopoulos, 2006). None of these single methods have proved to be completely efficient for OMW. Biological processes are within the most environmental friendly (no sludge production) and economic treatments, however are ineffective for OMW due to the high level of antimicrobial compounds in this effluent water (Hanafi et al., 2010). Because of this, high dilutions are usually required before biological treatments being applied (Justino et al., 2012; Mantzavinos and Kalogerakis, 2005). White-rot fungi (e.g. *Phanerochaete* spp. and *Pleurotus* spp.) have been pointed out as the most suitable organisms to treat OMW due to their capacity for degrading complex compounds like phenols which have a lignin-like structure (Morillo et al., 2009; Yesilada et al., 1999). With their extracellular ligninolytic enzymes, such as laccase, manganese peroxidase and lignin peroxidase, they are able to catalyze the degradation of lignin and the oxidation of persistent aromatic and halogenated compounds (Aytar et al., 2013; Baldrian, 2006). To overcome the limitations caused by the toxicity of OMW to biota, a dilution step or a pre-treatment may be required before the contact with fungi. The treatment of wastewaters combining chemical and biological processes has been reviewed in the literature (Mantzavinos and Kalogerakis, 2005; Scott and Ollis, 1995). Some of the most commonly applied and promising technologies for OMW are the advanced oxidation processes (AOPs), which were reported as effective in removing recalcitrant organic compounds, thus improving the biodegradability of this wastewater (Badawy et al., 2009; Michael et al., 2014; Stasinakis, 2008). AOPs are based on the *in situ* formation of highly reactive and oxidizing species, such as hydroxyl radicals, which accelerate the oxidation of a vast array of organic and inorganic pollutants with relatively low selectivity (Kestioğlu et al., 2005; Rizzo, 2011). Most of these methods consist in combinations of oxidizing agents (e.g. H_2O_2, O_3), ultraviolet or solar irradiation and catalysts (e.g. TiO_2, Fe_2O_3, ZnO). Among the different methods available, the Fenton's like reactions and heterogeneous photocatalysis with titanium dioxide, showed a great potential for the pre-treatment of organic wastewaters (Jamil et al., 2011; Justino et al., 2010). Badawy et al. (2009) compared photo-Fenton and TiO_2/UV processes as pre-treatment steps to enhance the biodegradability of OMW. The efficiency of both processes in terms of COD, TOC (total organic carbon), total phenolic compounds (TPC) and total suspended solids (TSSs) was shown by the reduction percentages of 87%, 84%, 97.44% and 98.31% attained for the four parameters, respectively, with photo-Fenton treatment and, of 68.8%, 67.3%, 40.2% and 48.9%, respectively, for the TiO_2/UV treatment. In parallel, the BOD_5/COD (BOD_5 – biochemical oxygen demand after 5 d) ratio increased for both treatments confirming the ability of these processes to enhance the biodegradability of the recalcitrant compounds present in the OMW (Badawy et al., 2009).

In the last few years nanoparticles (NPs) attracted great attention, since an improvement in the oxidation process is expected when they are applied as catalysts. In fact, the particle size, the band gap energy and the increased surface to volume ratio of the NPs, leads to more active surfaces for adsorption and also to higher redox potentials (Khin et al., 2012; Theron et al., 2008).

The oxidation processes showed great efficiency in the complete or almost complete mineralization of several recalcitrant organic compounds from the OMW. However, the complete mineralization is highly expensive (Machulek et al., 2013; Oller et al., 2011), but nanomaterials can greatly improve the chemical pretreatments (Han and Bai, 2009; Swetha et al., 2010).

Thus the main AIM of the present study was to test the combination of photocatalytic (nano-TiO_2/UV, nano-Fe_2O_3/UV, nano-TiO_2/H_2O_2/UV and nano-Fe_2O_3/H_2O_2/UV) and biological treatments with fungi (*Phanerochaete chrysosporium* and *Pleurotus sajor caju*), for improving the chemical characteristics of OMW and reducing its toxicity. the chemical pre-treatment was applied to diluted OMW (50%). However, this dilution was lower than those reported by previous studies (Jarboui et al., 2013; Ntougias et al., 2012). the efficiency of combined treatments was assessed through aromatics (270 nm), color reduction (465 nm), COD, TPC and ecotoxicity (Microtox®) reduction. to the best of our knowledge, this Is the first study applying biological treatment with fungi to OMW pre-treated by photocatalytic oxidation with nanomaterials.

2. Materials and methods

2.1. Olive oil mill wastewater source

The olive oil mill wastewater (OMW) sample tested was collected in an evaporation pond immediately after the annual olive oil production and was the by-product of a three-phase olive oil extraction process. OMW was stored in screw-caped glass flasks, at 4 °C, until the start of the experiments. Before use OMW was grossly filtered with a filter paper to remove the coarse particles under suspension and previously 50% diluted with distilled water. No further dilutions were made for the chemical or biological treatments. The major characteristics of the diluted effluent were: COD = 16.5 ± 0.6 g L^{-1}, TPC = 135.8 ± 7.2 mg L^{-1} and pH 4.5 ± 0.1.

2.2. Nano-catalysts and oxidant reagent

The two NPs used in this study were purchased as nanopowders. The titanium (IV) oxide anatase (TiO_2) was purchased from Sigma Aldrich, with particle size < 25 nm and 99.7% metal basis. The iron (III) oxide nanorods (Fe_2O_3) had an average particle size of 85 × 425 nm (d = 40–130 nm, l = 250–600 nm), a purity > 99% and was purchased from Nanostructured & Amorphous Materials Inc. (Houston, Texas, USA). Hydrogen peroxide (30%, analytical grade) was purchased from Fisheri Scientific. The toxicity of both NPs was previously tested for a battery of aquatic species (both freshwater and marine species) for concentrations up to 20 mg L^{-1}, as well for concentrations up to 2 g L^{-1} to the bacterium *Vibrio fischeri* (Nogueira et al., submitted). Both NPs were chosen because they showed a low toxicity in the range of concentrations tested.

2.3. Photocatalytic degradation

Photodegradation treatments were carried out using 200 ml of OMW in 250 ml glass beakers at room temperature (20 °C). Three replicates were prepared per treatment and control (raw effluent without chemical pre-treatment). The catalysts (nano-TiO_2 or nano-Fe_2O_3) were added to each beaker at a concentration of 1.0 g L^{-1}. The suspensions (effluent + catalyst) were placed in an orbital shaker (100 rpm) overnight to attain the adsorption–desorption equilibrium of the organic compounds on the surface of the catalyst. After this period the UV lamp (Spectroline XX15F/B, Spectronics Corporation, NY, USA, peak emission at 312 nm) was switched on, and left for 2 h. During irradiation, magnetic stirring was maintained to keep suspensions homogenous.

The influence of H_2O_2 was also investigated, by testing the systems nano-TiO_2/H_2O_2/UV and nano-Fe_2O_3/H_2O_2/UV with the addition of 17.0 g L^{-1} of H_2O_2 before UV irradiation and in the same conditions described above. Additional replicates were prepared for this purpose (three per treatment). The catalyst and oxidant concentrations were selected based on a previous study from our team (Nogueira et al., submitted, data not shown). After