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Combining fungal bioremediation and ozonation for rinse wastewater treatment

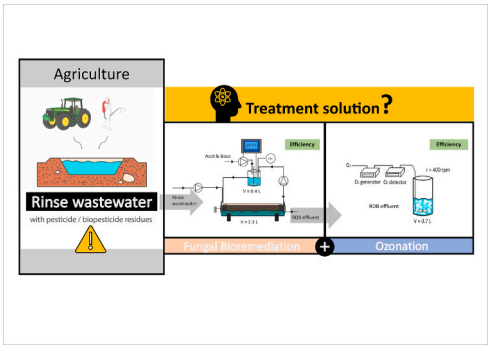
Eduardo Beltrán-Flores [a], Paqui Blánquez [a], Ana M. Gorito [b,c], Montserrat Sarrà [a,*], Adrián M. T. Silva [b,c]

[a] Departament d'Enginyeria Química Biològica i Ambiental, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[b] LSRE-LCM – Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[c] ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

HIGHLIGHTS

- Agricultural rinse wastewater was successfully treated by different technologies.
- Fungal bioremediation was better in terms of toxicity, formation of TPs and cost.
- Ozonation was superior in removing pesticides, colour, organic matter and bacteria.
- The treatment train integrated the advantages of both techniques.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, agricultural rinse wastewater, which is produced during the cleaning of agricultural equipment and constitutes a major source of pesticides, was treated by fungal bioremediation and ozonation, both individually and combined in a two-stage treatment train. Three major pesticides (thiacloprid, chlortoluron, and pyrimethanil) were detected in rinse wastewater, with a total concentration of 38.47 mg C L^{-1}. Comparing both technologies, ozonation in a stirred reactor achieved complete removal of these pesticides (720 min) while proving to be a more effective approach for reducing colour, organic matter, and bacteria. However, this technique produced transformation products and increased toxicity. In contrast, fungal bioremediation in a rotating drum bioreactor attenuated toxicity levels and did not produce such metabolites, but only removed approximately 50 % of target pesticide - hydraulic retention time (HRT) of 5 days - and obtained worse results for most of the general quality parameters studied. This work also includes a preliminary economic assessment of both technologies, revealing that fungal bioremediation was 2 times more cost-effective than ozonation. The treatment train, consisting of a first stage of fungal bioremediation followed by ozonation, was found to be a promising approach as it synergistically combines the advantages of both treatments, achieving high removals of pesticides (up to 100 %) and transformation products, while reducing operating costs and producing a

* Corresponding author.
E-mail address: montserrat.sarra@uab.cat (M. Sarrà).

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biodegradable effluent. This is the first time that fungal bioremediation and ozonation technologies have been compared and combined in a treatment train to deal with pesticides in agricultural rinse wastewater.

1. Introduction

Pesticides comprise a broad group of organic compounds essential for sustaining the current lifestyle of our societies. These compounds, together with fertilizers and the use of appropriate machinery, improve crop varieties and enhance agricultural productivity by reducing losses caused by the proliferation of weeds, pests, and diseases (Aktar et al., 2009). Despite that, their indiscriminate use can cause severe and irreversible damage to the integrity of the environment and human health. Some studies have associated exposure to these compounds with the development of various human diseases, such as cancer, asthma, and diabetes (Huang et al., 2019; Stoleski et al., 2019). Additionally, interactions between pesticides can trigger synergistic mechanisms, leading to unpredictable toxicological effects (Hernández et al., 2017).

In response to this concerning issue, the European Commission (EC) has promoted the implementation of Integrated Pest Management (IPM) through the 2009/128/EC Directive on the sustainable use of pesticides (European Commission, 2009), which calls for the adoption of sustainable agricultural practices. These principles include cultivation techniques (crop rotation, balanced fertilization, *etc.*), pest monitoring, use of biopesticides, selection of low-risk pesticides and dosages, and hygiene measures. Concerning hygiene measures, they should encompass the management of rinse wastewater (RWW), generated during the washing of agricultural machinery and equipment that has been in contact with pesticides. In this regard, some studies and guidelines recommend the reuse of the RWW as a phytosanitary product in the same agricultural fields (EPA, 2012; Life aquemfree, 2018; Shukla et al., 2001). Nonetheless, this strategy is unfeasible for large farms that typically produce significant RWW volumes. In this case, RWW usually accumulates in large collection ponds where they are concentrated by natural evaporation, thus generally presenting high toxicity and pesticide content (Kuo and Regan, 1999). This is a particular type of wastewater, whose treatment is scarcely documented. Therefore, the development of treatment approaches specifically designed to address this problem is imperative, contributing to reducing toxicity and, eventually, increasing RWW reuse.

Among the chemical oxidation technologies, some of them conceptually known as advanced oxidation processes (AOPs), ozonation stands out as one of the most mature and consolidated options for degrading organic pollutants, including micropollutants, in wastewater matrices. Ozone (O_3) is a molecule with high oxidative capacity and disinfectant potential (Von Gunten, 2003). Molecular O_3 can directly attack organic pollutants (mainly at low pH), or it can generate highly reactive species such as hydroxyl radicals (HO•, mainly at high pH) that can indirectly oxidize them (Sharma et al., 2018). Nevertheless, this technology has two main drawbacks that currently limit its full-scale application: the possible formation of transformation products and high operating costs. These limitations could be particularly critical in the case of RWW, given their relatively high content of pesticides and organic matter, which can lead to increased formation of by-products and the need for higher O_3 dosages, along with respective costs.

Alternatively, bioremediation is considered a safe, low-cost, and sustainable technology that uses microorganisms, plants, and enzymes to mitigate pollution (Harms et al., 2011; Saravanan et al., 2021). Among other microorganisms, white rot fungi (WRF) are gaining increasing attention due to their complex enzyme system, composed of both extracellular enzymes (mainly lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase) and the intracellular system known as cytochrome P-450, which is capable of degrading a wide range of pollutants (Asgher et al., 2008). This feature is particularly advantageous for treating RWW, as these waters generally contain a broad spectrum of pesticides (Beltrán-Flores et al., 2023a). Other microorganisms, such as bacteria, are very efficient at removing certain pollutants (Saravanan et al., 2023), but they may face challenges in treating RWW due to their specific biochemical degradation pathways. In fact, conventional activated sludge treatments are ineffective in removing recalcitrant compounds such as pesticides (Sutton et al., 2019). In addition, the slow metabolism of fungi makes bioremediation with these microorganisms particularly applicable to relatively small volumes of water, which is typical in RWW (*e.g.*, compared to urban or hospital wastewater). In comparison to other physicochemical processes, bioremediation is usually considered more environmentally friendly, *i.e.*, this approach uses biodegradable biomass instead of synthetic chemical reagents, and more cost-effective (Harms et al., 2011), but some of these aspects have not yet been sufficiently studied in fungal bioremediation.

Another option for RWW treatment is the implementation of a treatment train. Treatment trains can synergistically combine the advantages of multiple technologies, often achieving better results than those obtained by each treatment applied alone. In this regard, AOPs can be employed as pre-treatment to oxidize biologically recalcitrant compounds, making wastewater more biodegradable (Mansour et al., 2014). These pre-treatments typically aim for low mineralization of pollutants to avoid high operating costs. Subsequently, a biological stage can be implemented to eliminate the potential transformation products (TPs). Nonetheless, several studies have also explored the strategy in the opposite direction, *i.e.*, applying a bioremediation treatment first to reduce the pollutant load, followed by an AOP stage to complete its removal or even mineralization in less time, and consequently, lower costs (Oller et al., 2011). The latter alternative could be more appropriate for treating RWW, which typically contains high concentrations of pesticides and organic matter.

The objective of this study was to investigate the treatment of RWW using ozonation, fungal bioremediation, and the combination of both technologies in a treatment train. Therefore, this study explores various specific approaches for the treatment of RWWs, whose physicochemical properties complicate their remediation by conventional methods. The performance of each strategy was compared in terms of pesticide elimination, generation of TPs, overall RWW quality, and economic costs. The conclusions drawn from the comparative study have the potential to drive the development and implementation of RWW treatment solutions in full-scale scenarios.

2. Materials and methods

2.1. Fungal strain and reagents

The WRF *T. versicolor* ATCC 42530 was purchased from the American Type Culture Collection. *T. versicolor* was maintained on malt extract (2 % w/v) at 25 °C and routinely subcultured every 30 days. A mycelial suspension of *T. versicolor* was prepared as previously described elsewhere (Blánquez et al., 2004). *T. versicolor* immobilized on *Q. ilex* wood chips was prepared as described by (Beltrán-Flores et al., 2021). *Q. ilex* wood chips presented a porosity of 62.4 ± 0.5 %, a wet to dry weight ratio (WW/DW) of 2.5 ± 0.1, and real and apparent densities of 0.27 ± 0.01 g mL^{-1} and 0.89 ± 0.02 g mL^{-1}, respectively (calculations are shown in the Supplementary Material section).

Acetonitrile, formic acid, and sulfuric acid were purchased from Merck (Darmstadt, Germany). Analytical standards of the pesticides thiacloprid (THIA), chlortoluron (CHLOR), and pyrimethanil (PYRI) were purchased from Sigma-Aldrich (Barcelona, Spain). These chemicals and others used for *T. versicolor* subculture were of high purity

grade.

2.2. Agricultural rinse wastewater

RWW was sampled in August 2021 from an artificial pond designed to collect wastewater produced after washing spraying equipment and agricultural machinery in the Sustainable Plant Protection program of the Institute of Agrifood Research and Technology (IRTA) in association with the Mas Badia Foundation (La Tallada d'Empordà, Spain). Wastewater was stored in the fridge at 4 °C until use. The physicochemical characteristics of the RWW are summarized in Table S1 (Supplementary Material).

2.3. Fungal treatment in a rotating drum bioreactor (RDB)

Fungal bioremediation was performed in a rotating drum bioreactor (RDB), which has been used in our previous works, and proven to be a promising candidate for agricultural wastewater treatment, as this reactor has exhibited high pesticide removal capacity and its design has been adapted for *in-situ* application in agricultural fields (Beltrán-Flores et al., 2023a, 2022). In the present work, two RDBs were operated in parallel, the experimental reactor (*E*-RDB), which was assembled with wood initially colonized by *T. versicolor*, and the control reactor (C-RDB), which only contained wood. The E-RDB and the C-RDB were operated for 67 and 53 days, respectively, with a hydraulic retention time (HRT) of 5 days. The inner tube rotated one and a half turns every 24 h to alternate the submerged biomass fraction. A total of 545 g dry weight (DW) of colonized wood was introduced inside the inner tube. An external recirculation loop (4.7 L day^{-1}) was required for pH adjustment and dissolved oxygen (DO) measurement, which were performed in a recirculation tank (≈0.4 L). The total volume treated was 2.7 L. The RWW was previously adjusted to pH 4.5 and then automatically controlled throughout the treatment by adding either 1 M hydrochloric acid (HCl) or sodium hydroxide (NaOH) inside the recirculation tank. Aeration was introduced at the reactor inlet from day 28 onwards through a diffuser at 0.1 NL/min. DO was measured by using an OXROB10 robust oxygen probe coupled to a FireSting-PRO (4 channels) fiber-optical multi-analyte meter (Pyroscience, Germany). Samples of 3 mL were withdrawn throughout the treatment to measure the concentration of the pesticides (Section 2.5.1) and laccase activity (Section 2.5.4). The effluent was stored in collection tanks for subsequent analysis and treatment by ozonation.

2.4. Ozone treatment in a stirred reactor

2.4.1. Experiments with spiked ultrapure water

Ultrapure water (0.7 L) was spiked with 16 mg L^{-1} THIA, 34 mg L^{-1} CHLOR, and 46 mg L^{-1} PYRI and treated by ozonation for 480 min in a stirred semicontinuous reactor (*i.e.*, the liquid phase is static (batch), and the gas phase (O_3) is dynamic, flowing through the liquid). This reactor had three ports at the top part: an O_3 inlet, a gas outlet, and a sample port. The RWW was magnetically stirred at 400 rpm. O_3 was generated from pure oxygen in a BMT 802× O_3 generator (BMT Messtechnik, Germany) and was introduced into the reactor through a ceramic diffuser at a constant concentration (50 g Nm^{-3}) and flow rate (150 Ncm^3 min^{-1}). A BMT 964 O_3 analyser (BMT Messtechnik, Germany) was used to monitor the O_3 concentration in the outlet gas stream. The gaseous outlet of the reactor was connected to a bottle with a potassium iodide solution to remove the O_3 leaving the reactor.

Aliquots of 1 mL were withdrawn from the reactor at specific times (depending on the experiment) for further analysis of pesticides and concentrations of TPs. In addition, at the end of the experiment, liquid samples were taken for the analysis of colour, chemical oxygen demand (COD), biological oxygen demand (BOD_5), dissolved organic carbon (DOC), toxicity, and heterotrophic plate counts (HPCs). The treatment was performed at room temperature (approximately 18 °C). The trials were performed in triplicate.

2.4.2. Experiments with RWW and RDB effluent

The original RWW and the RDB effluent (0.7 L) were treated by ozonation until complete degradation of the three studied pesticides (720 min) in the stirred glass reactor described in Section 2.4.1. Aliquots of 1 mL, or 8 mL when analysing DOC, were withdrawn from the reactor at specific times (depending on the experiment) for further analysis of pesticides and concentrations of TPs. As described for ultrapure water, at the end of the experiment, liquid samples were taken for the analysis of colour, COD, BOD_5, DOC, toxicity, and HPCs. Two additional experiments were conducted for the treatment of the RDB effluent in which NaOH and methanol (MeOH) were added to evaluate their effect on the O_3 treatment performance. As a result, the RDB effluent was also treated by ozonation at a higher initial pH of 7.6, which can favour the production of $HO^{\bullet}$, and with 2 % (v/v) MeOH which, conversely, can act as a radical scavenger. Again, samples were taken at specific times (depending on the experiment) to measure the pesticides and concentrations of TPs.

2.5. Analytical techniques

2.5.1. Identification and quantification of pesticides

The analysis of pesticides was performed in two steps: (1) pesticides were identified by high-performance liquid chromatography coupled to time-of-flight mass spectrometry (HPLC-qTOF-MS); (2) and then quantified by HPLC with ultraviolet detection (HPLC-UV). The analysis methodology has been described in a recent publication (Beltrán-Flores et al., 2023a), but it is also available in the Supplementary Material Section.

2.5.2. Identification and quantification of TPs

The concentrations of oxalic, oxamic, and maleic acids were quantified as described elsewhere (Torres-Pinto et al., 2019). In brief, these aliphatic acids were measured by using a Hitachi Elite Lachrom HPLC equipped with a UV–vis detector and a 5 mM sulfuric acid (H_2SO_4) mobile phase through an Alltech OA-1000 Organic Acids column (300 mm). The eluent was pumped at a constant flow rate of 0.5 mL min^{-1}. The sample loop volume and detection wavelength were set at 30 μL and 200 nm, respectively. The limit of detection (LOD) in ultrapure water was 0.20 and 0.06 mg L^{-1} for oxalic and oxamic acids, respectively, and in the RWW the LOD was 0.5 ppm for both oxamic and maleic acids. Under these conditions, the retention times were approximately 5.90, 8.50, and 9.10 min for oxalic, maleic, and oxamic acids, respectively.

2.5.3. Vibrio fischeri bioluminescence inhibition test (Microtox® test)

A toxicity test was performed using the acute toxicity bioassay kit from Modern Water (London, UK). In brief, the test is based on the attenuation of *Vibrio fischeri* bioluminescence after 5 and 15 min of exposure to selected dilutions of the samples (original and treated RWW effluents), previously adjusted to pH 7. Toxicity was expressed as toxicity units (TU). Samples were analysed in triplicate.

2.5.4. Laccase activity

Laccase activity was measured at the RDB outlet and was determined through the absorbance changes induced by the oxidation of 2,6-dymetoxyphenol (DMP) and expressed in activity units per litre (UA·L^{-1}), as described elsewhere (Wariishi et al., 1992).

2.5.5. RWW characterization

Analyses of the general characteristic parameters of the RWW, namely conductivity, colour, COD, BOD_5, ammonia, total suspended solids (TSS), volatile suspended solids (VSS), HPCs, DOC, and anions were conducted according to the information of the Supplementary Material section.

3. Results and discussion

3.1. RWW characteristics

THIA, CHLOR, and PYRI were detected as the major pesticides at concentrations of approximately 10, 25, and 28 mg L^{-1}, respectively. This study not only focuses on pesticide removal but also analyses conventional parameters to evaluate the final effluent quality. By analysing the physico-chemical parameters of RWW (Table S1) it was perceptible the high content of intrinsic organic matter detected in terms of COD (4263 ± 85 mg O_2 L^{-1}) and DOC (1527 ± 35 mg L^{-1}) when compared to other agricultural and urban wastewater. As a consequence of the eventual decomposition of this organic matter, relatively high levels of ammonium and bacteria counts were also detected (Beltrán-Flores et al., 2021; Metcalf and Eddy, 2003).

3.2. Fungal treatment in the RDB

3.2.1. Sorption contribution

The RWW was treated for 67 days in the E-RDB and 53 days in the subsequent C-RDB (Fig. 1). In the non-inoculated C-RDB, the removal was mainly ascribed to the sorption of these pesticides onto the wood. In this regard, a recent publication showed that the sorption of THIA and CHLOR were 66 % and 62 %, respectively, while the remaining removal (16 % and 13 %, respectively) was attributed to biological activity (Beltrán-Flores et al., 2023a). Considering the differences between the elimination results of the two reactors (Fig. 1), the contribution of sorption was found to be decisive during the treatment. Although most pesticides were mainly removed by sorption, previous studies have shown that *T. versicolor* can degrade sorbed pesticides in a subsequent solid-phase treatment (Beltrán-Flores et al., 2023a, 2022)

3.2.2. Total removal of pesticides

DO was measured with the aid of a standard oxygen probe in the recirculation tank, which remained above 30 % throughout the treatment, thus biomass was initially assumed to work under non-limiting conditions. However, from day 28th onwards, another DO probe was used, which was thin enough to be inserted directly into the channel. This probe revealed a very low oxygen level within the channel (<5 %), which indicated the submerged biomass had probably been working under limiting conditions (Beltrán-Flores et al., 2023b). For this reason, aeration was incorporated into the channel, maintaining the DO level near saturation (*i.e.*, 100 %) for the remaining treatment period.

After the addition of aeration, a water loss by evaporation of approximately 1 cm in height (approximately equivalent to 0.3 L concerning the initial 2.7 L) was observed, which could have caused the concentration of the pesticides and therefore would explain the decrease in removal yield from day 28 onwards for both the E-RDB and the C-RDB (Fig. 1). However, pesticide removal in the E-RDB increased from day 39th onwards, even surpassing the initial elimination yields, when wood sorption capacity was maximum. The average removals during the entire treatment period in the E-RDB were 45 % THIA, 49 % CHLOR, and 53 % PYRI, corresponding to a total elimination in terms of DOC of 50.43 % (19.40 mg C L^{-1}). Similar removals were reported for two other pesticides, diuron (up to 54 %) and bentazon (up to 48 %), in the same RDB, with sorption being the predominant elimination mechanism (Beltrán-Flores et al., 2022). In a recent publication, higher THIA and CHLOR removals (82 % and 75 %, respectively) were obtained in an RDB, but in this case using a longer HRT of 17 days (Beltrán-Flores et al., 2023a).

The results on the elimination of target pesticides are in line with those reported in previous WRF bioremediation experiments. To the best of the authors' knowledge, no prior research on the degradation of THIA by WRF has been reported, except for the one mentioned previously

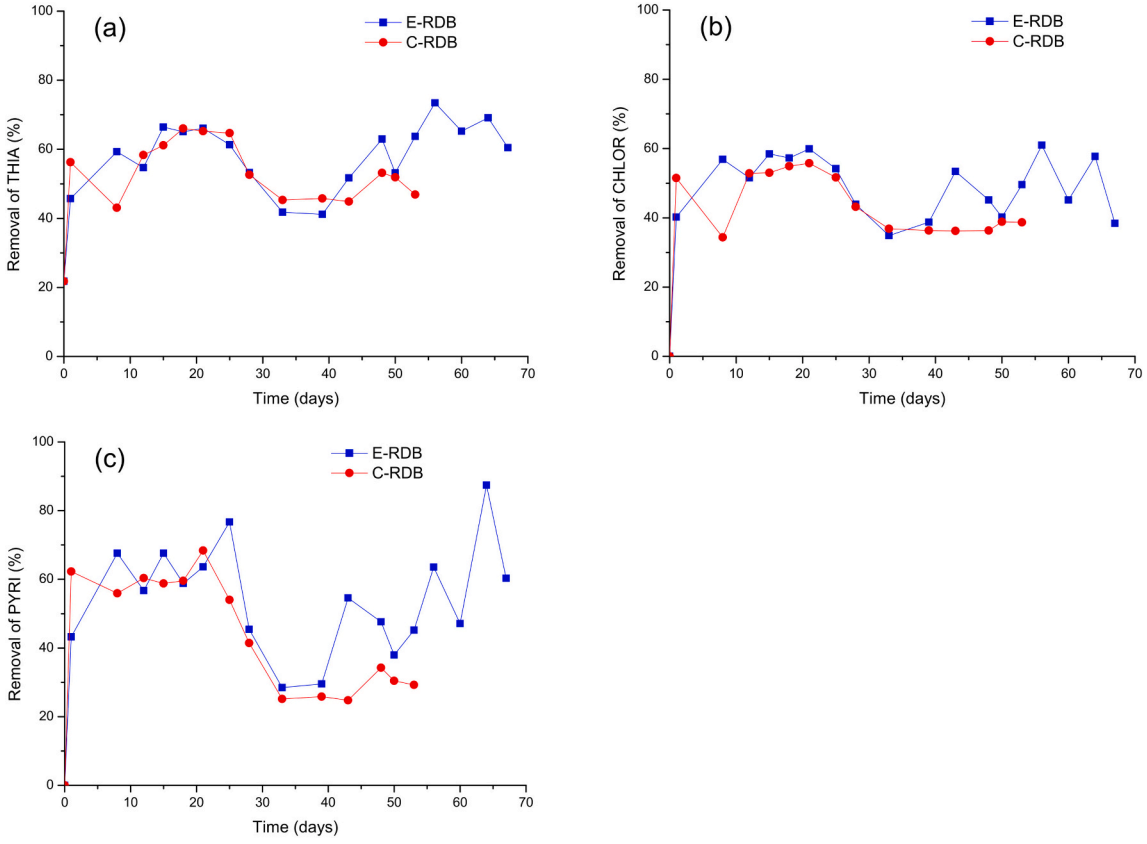

Fig. 1. THIA (a), CHLOR (b) and PYRI (c) removal profiles by *T. versicolor* immobilized on *Q. ilex* with an HRT of 5 days in the RDB. Blue squares and red circles are the removals by the experimental (E-RDB) and control (C-RDB) reactors, respectively.

(Beltrán-Flores et al., 2023a). Nevertheless, previous studies have documented WRF bioremediation of compounds closely related to THIA, both in terms of family type and molecular structure, such as imidacloprid and acetamiprid. Various studies conducted in Erlenmeyer flask have demonstrated the ability of different WRFs to degrade these types of pesticides. Recently, Zhu et al. (2023) systematically explored the enzyme-mediated biotransformation mechanism of imidacloprid (10 mg L^{-1}) by the WRF *Phanerochaete chrysosporium*, achieving a 93.5 % bioconversion after 6 days. Regarding acetamiprid, removals of up to 51 % (22 mg L^{-1} initial concentration) and 45 % (2 mg L^{-1} initial concentration) over treatment periods ranging from 15 to 20 days have been reported for *P. chrysosporium* and *Phanerochaete sordida YK-624*, respectively (Wang et al., 2019, 2012). Furthermore, in an air-pulse fluidized bioreactor experiment using *T. versicolor* pellets, degradation yields of 20 % and 65 % were achieved for imidacloprid and acetamiprid (4 mg L^{-1} of each one), respectively, after 7 days of operation (Hu et al., 2022).

In another study, the degradation of CHLOR (100 mg L^{-1}) by different WRF was assessed in liquid synthetic medium for 3 days, resulting in removals of 15 % by *Lentinus tigrinus*, 18 % by *Ceriporiopsis subvermispora*, 33 % by *Phlebia radiata*, 33 % by *Inonotus hispidus*, 34 % by *Trametes sp.* and 71 % by *Bjerkandera adusta* (Khadrani et al., 1999). The degradation of PYRI (20 mg L^{-1} initial concentration) by *T. versicolor* exceeded 60 % in the presence of a suitable mediator after 10 h of incubation (Jin et al., 2016). Therefore, these studies support the findings of the present experiments, as they have previously demonstrated the ability of WRF to remove these types of compounds and achieve comparable degradation activity.

In future research, several strategies could be implemented to enhance degradation efficiency. These strategies include extending the HRT, for example to 17 days, as previously reported (Beltrán-Flores et al., 2023a), initiating aeration from the beginning of the treatment, incorporating a preliminary stage for fungal acclimatization, and employing a consortium of WRF. In this regard, the fungal consortium could be comprised of strains that have previously exhibited higher degradative activity for the pesticides detected in each RWW. In this case, a mixture of *B. adusta*, *P. chrysosporium*, and *T. versicolor* would be a reasonable possibility. Additionally, synergistic cooperation between bacteria and fungi for pesticide degradation has also been explored previously, and future studies in this field could consolidate the reactor microbial consortium and improve treatment performance (Purnomo et al., 2020, 2017).

Laccase activity was also monitored throughout the treatment and is explained in the Supplementary Material section. Other characterization parameters were also analysed and are presented in the comparative study in Section 3.6. Microbial community analysis is not presented in this study, as it has been previously addressed for these RWW in a recent publication (Beltrán-Flores et al., 2023a).

3.3. Ozonation in a stirred reactor

Before treating the RWW, an experiment was conducted with ultrapure water spiked with the target pesticides to evaluate their degradability by ozonation. Subsequently, the RWW, in which these pesticides were intrinsically present, was treated. The initial concentrations at which the ultrapure water was spiked were slightly higher (although of the same order of magnitude) than those found in the RWW (Section 3.1). The degradation profiles of the three model pesticides in ultrapure water are shown in Fig. 2. All three pesticides were completely removed (below the LOD) by ozonation: after 240 min for THIA, 180 min for CHLOR, and 120 min for PYRI. In fact, after 20 min of ozonation, THIA, CHLOR, and PYRI have already reached removals of 39, 54, and 88 %, respectively. These results are in line with previous studies, in which different AOPs had already been able to remove these pesticides. For instance, THIA spiked in groundwater (0.1 mg L^{-1}) was treated by ozonation for 20 min, achieving eliminations around 40 %, 50 %, and

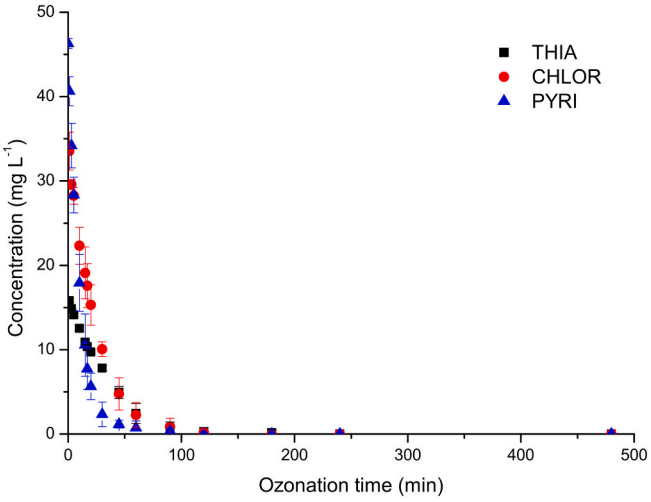

Fig. 2. Evolution of THIA, CHLOR, and PYRI for ozonation (O_3) with an inlet concentration of 50 g O_3 Nm^{-3} and flow rate of 15 Ncm^3 min^{-1} in the gas phase in experiments performed with spiked ultrapure water. Values are means ± standard deviation for triplicate samples.

65 % with doses of 0.5, 1.0, and 1.5 mg O_3 mg DOC^{-1} respectively (Guo et al., 2020). Other authors (Benitez et al., 2007) applied O_3 at constant 40 L h^{-1} for the treatment of CHLOR spiked in some natural water systems (1 μM, corresponding to 0.21 mg L^{-1}), reporting eliminations over 60 % and 100 % for O_3 concentrations of $1{\cdot}10^{-5}$ M and $3{\cdot}10^{-5}$ M, respectively. In the case of PYRI, this pesticide was found to be highly reactive with O_3 (Ochir et al., 2021). An equivalent molar amount of O_3 relative to PYRI spiked in rainwater (2 μM, corresponding to 0.4 mg L^{-1}) was supplied, PYRI being completely removed after 20 min.

Fig. 3(a) shows the results of pesticide concentrations during the ozonation treatment of the original RWW. As occurred with the spiked ultrapure water, all three pesticides were completely removed using single ozonation. However, a significantly longer treatment period of up to 720 min was needed in the case of RWW [Fig. 3(a)], instead of <240 min when treating ultrapure water with a higher initial concentration of these pesticides (Fig. 2). This slower degradation can be attributed to the complex matrix of the RWW, which contained a considerable amount of natural organic matter (Table S1) that probably interacted with O_3 and/or its radicals by acting as a scavenger, decreasing the treatment efficiency.

On the other hand, Fig. 3(b) shows the results of the pesticide concentrations during the ozonation treatment of the fungal-treated effluent. The effluent produced throughout the fungal treatment in the RDB was stored in an accumulation tank to be subsequently treated by ozonation. For this reason, the initial concentrations in Fig. 3(b) are lower than those in Fig. 3(a). Despite the lower initial pesticide concentrations, the same treatment time (720 min) used for the original RWW was required to completely remove all target pesticides. This result may be due to the fact that during the RDB treatment, despite the decrease in pesticide concentration, some soluble compounds were extracted from the wood, increasing the content of natural organic matter (Table 1). Accordingly, a comprehensive kinetic study is presented in the Supplementary Materials section. This finding has also been described in previous studies (Beltrán-Flores et al., 2023a, 2022). This phenomenon has been reported previously, for example, (Liu et al., 2020) obtained different removal rates of some trace organic compounds (including pesticides such as alachlor, atrazine, and pentachlorophenol) depending on the water matrices and their scavenger content. Additionally, experiments studying the effect of pH increase or adding MeOH are described in the Supplementary Material section, being possible to understand that the simultaneous removal of THIA, CHLOR, and PYRI was slightly favoured by the presence of $HO^{\bullet}$ radicals.

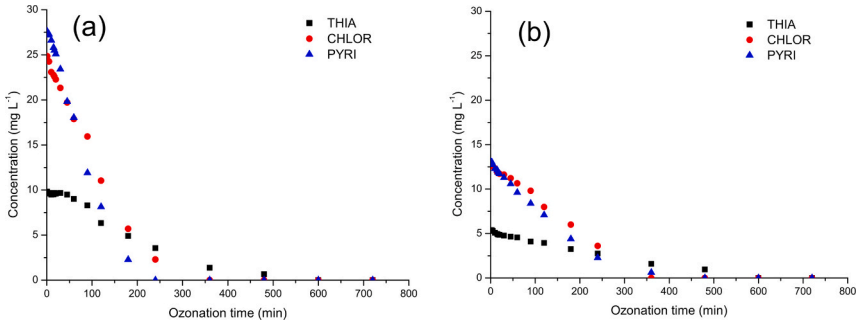

Fig. 3. Evolution of THIA, CHLOR, and PYRI for ozonation (O_3) with an inlet concentration of 50 g O_3 Nm^{-3} and flow rate of 15 Ncm^3 min^{-1} in the gas phase in experiments performed with (a) the original RWW and (b) the fungal-treated RWW.

Table 1
Characterization parameters of the original RWW and the differently treated effluents.

Sample	pH	Colour (abs)	COD (mg O_2 L^{-1})	BOD_5 (mg O_2 L^{-1})	BOD_5/COD ratio	DOC (mg C L^{-1})	Toxicity (TU)	HPCs (CFU mL^{-1})
Original RWW	7.64	0.713	4263	135	0.03	1527	13.53	$2.10{\cdot}10^5$
Biorem. effluent	4.50	1.068	11,180	1588	0.14	2981	13.49	$6.82{\cdot}10^5$
O_3 effluent	4.38	0.811	2944	215	0.07	1072	16.37	<LOQ
Effluent from Biorem. + ozonation	3.12	0.654	10,030	3050	0.30	2656	12.06	<LOQ

Note: LOQ is the limit of quantification. In all cases, standard deviations below 5 % toxicity (triplicate) and 2 % HPCs (duplicate) were obtained.

3.4. Technology combination: Pesticide removal

Fig. 4 shows the removal of the studied pesticides (in terms of DOC) achieved by direct ozonation of the original RWW (blue squares) and by the treatment train, *i.e.*, fungal bioremediation (in the RBD) followed by ozonation (green circles), during the ozonation period. The results of the treatment train (bioremediation + ozonation), started with an elimination value of 50.43 %, which corresponds to the average elimination achieved during the fungal treatment in the RDB (HRT = 5 days, treatment period = 67 days). This value is in line with those previously reported in the literature (Beltrán-Flores et al., 2022). Note that to achieve the same level of removal, direct ozonation required approximately 103 min. Comparing both curves, a positive contribution of bioremediation can be deduced during the first 168 min of the ozonation treatment when approximately 77 % removal was reached. From this period onwards, the removal efficiencies were practically identical in both cases, until reaching 100 % elimination. Therefore, this study suggests that the treatment train would only be appropriated when a pesticide removal of <77 % is required; otherwise applying O_3 alone would be preferable to reduce the complexity of the treatment system. O_3 alone is an excellent option to achieve high removal efficiencies in a very short time, as reported elsewhere (Gorito et al., 2021); however, several factors should be considered to facilitate the selection between direct ozonation or the treatment train, namely, TPs, toxicity, organic matter content, and operating costs.

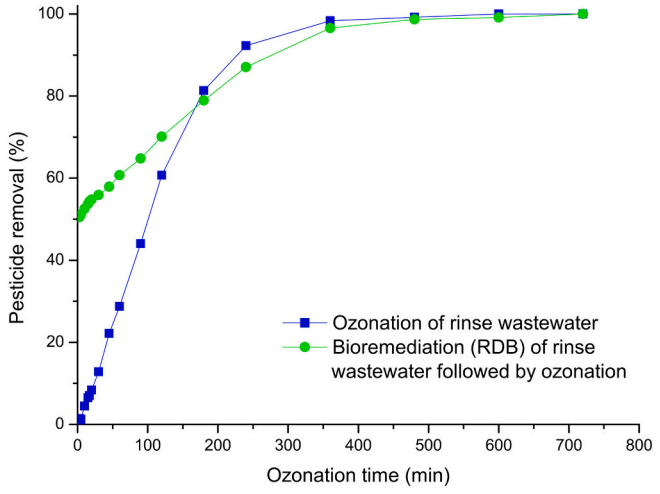

Fig. 4. Pesticide removals achieved by ozonation applied to the original RWW (blue squares) and the fungal-treated effluent (green circles). The results only correspond to the ozonation period.

3.5. Technology combination: Transformation products

Ozonation is a process that is well known to generate TPs, including different types of low-molecular-weight carboxylic acids that tend to accumulate in solution not enabling a high mineralization degree (Moreira et al., 2016). In this regard, three ozonation TPs (oxalic, oxamic, and maleic acids) were detected and monitored in the present study. Fig. 5 shows the evolution of the concentrations of the TPs during the ozonation experiments with spiked ultrapure water. Oxalic and oxamic acids (at quite similar concentrations) were produced, while no maleic acid was detected. Oxalic acid has been identified as one of the most typical TPs in the ozonation of several organic compounds. Oxamic acid is also common in the ozonation of organic compounds containing nitrogen functional groups and is considered to be an even more refractory TP than oxalic acid (Faria et al., 2008).

The same analysis of the TPs was performed for the RWW treated by fungal bioremediation only (results not shown). Neither oxamic nor maleic acids were produced by fungal bioremediation. Maleic acid has been proposed as a potential metabolite in the degradation pathway of some pesticides by *T. versicolor*, although it has not yet been detected (Hu et al., 2022). No literature was found on the production of oxamic acid. Regarding oxalic acid, it is well known that WRF secrete oxalic acid during its secondary metabolism to degrade cellulose (Dutton et al., 1994), but this compound was not detected in the experiments with RWW most probably due to the interferences with the matrix.

In the case of ozonated RWW, the TPs detected were oxamic and maleic acids (Fig. 5). Although oxalic acid was most probably also generated by ozonation of the RWW, it could not be detected in the present work. Probably, some interference occurred in the chromatographic analysis of RWW since it was detected in the trials with

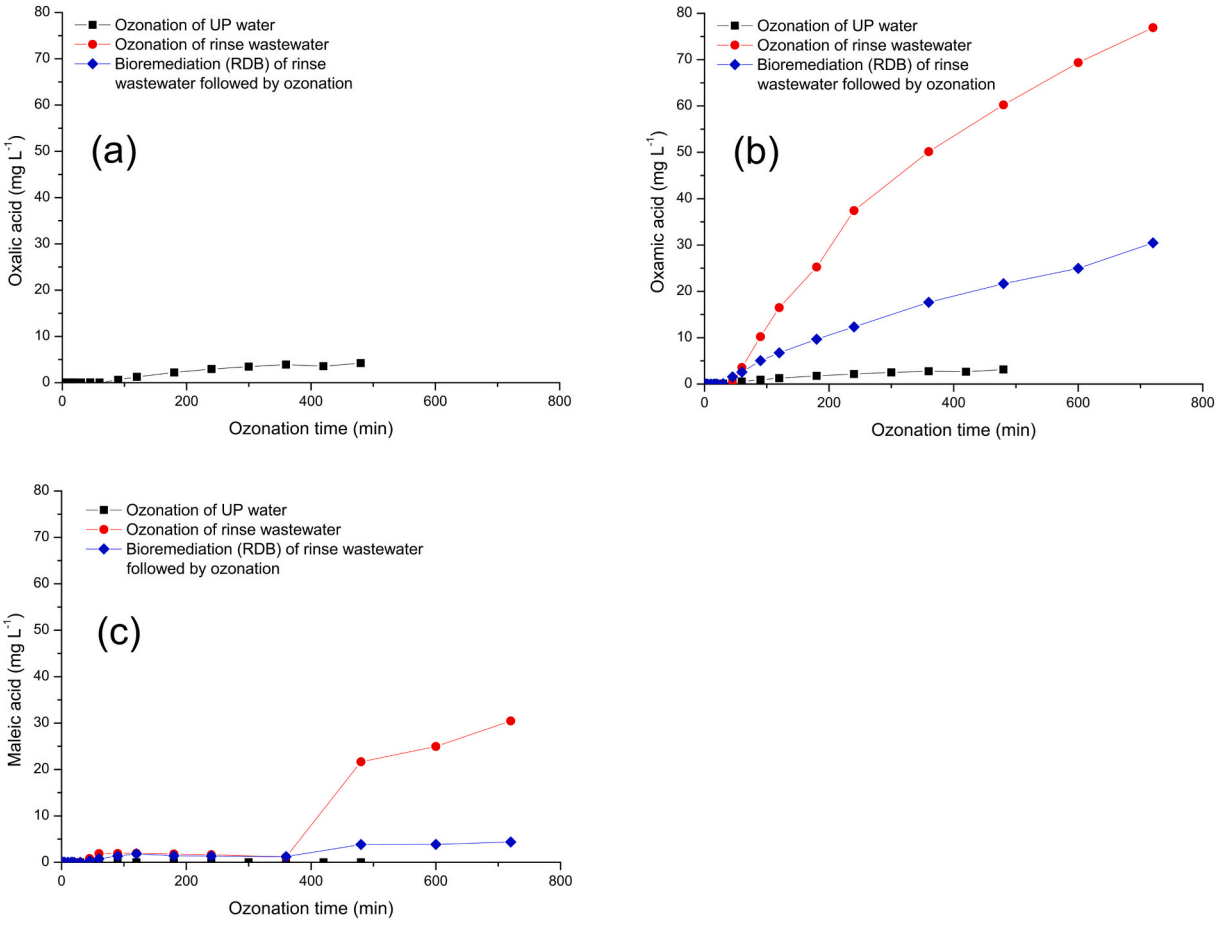

Fig. 5. TPs generated during the ozonation stage: (a) oxalic, (b) oxamic and (c) maleic acids. In subfigure (a) oxalic acid is not shown in either the RWW or the RWW previously treated by bioremediation because it was not detected probably because of matrix interference.

ultrapure water. It is interesting to observe that maleic acid was produced by ozonation of the RWW, which was not detected in the treatment of spiked ultrapure water, and thus its occurrence was attributed not to the oxidation of the target pesticides, but to the degradation of other organic compounds contained in the matrix. As expected, the matrix also has an important role in oxamic acid production, which showed considerably higher concentrations in the ozonation of RWW (approximately 60 mg L^{-1} in RWW *vs* 3 mg L^{-1} in ultrapure water, 500 min).

Fig. 5 also shows that the introduction of a fungal bioremediation process before the ozonation stage significantly reduced the production of oxamic and maleic acids. This favourable result was attributed to the powerful enzyme system of *T. versicolor*, which can degrade a wide range of organic compounds, thus limiting the production of potential metabolites (Mir-Tutusaus et al., 2018). Therefore, these results support the use of the treatment train instead of direct ozonation to reduce the production of the studied TPs. A final stage of conventional activated sludge could also be applied, either *in-situ* in the agricultural field or in a wastewater treatment plant (WWTP), to achieve the complete elimination of these TPs (Nakamura et al., 2004).

3.6. Technology combination: Study of other characteristic parameters

Table 1 shows the results of other key parameters of the original RWW and the effluent from each treatment. The organic matter content was drastically increased during the bioremediation stage, mainly attributed to the release of organic compounds from the wood. In contrast, ozonation was able to reduce the COD, especially in the case of direct ozonation of RWW (from 4263 to 2944 mg O_2 L^{-1} in 720 min). Similar conclusions were obtained when analysing the DOC, which increased during the bioremediation process and decreased considerably with ozonation, particularly with direct ozonation of RWW (from 1527 to 1072 mg C L^{-1} in 720 min). Ozonation has already been shown to degrade parent compounds partially or even completely, but it is often unable to fully mineralize them due to the formation of various types of TPs, namely the above-mentioned low-molecular-weight carboxylic acids (Moreira et al., 2015). Table 2 shows the theoretical contributions of the pesticides and the TPs to the total DOC, which was experimentally measured. Regarding the results of degradation with ultrapure water, ozonation completely degraded the pesticides after 4 h of treatment, but not the studied TPs even after 8 h. In the case of the RWW, the contribution of the pesticides and TPs detected to total DOC was very low, indicating the existence of significant unidentified organic matter.

The colour (Table 1) was measured by absorbance, which increased during the bioremediation treatment. This increase was attributed to the extraction of soluble organic compounds from the wood, some of which give the characteristic dark colour to *Q. ilex* wood (Beltrán-Flores et al., 2023a, 2022). This effluent was appreciably clarified by ozonation, which is a well-known process for removing colour from the medium (Mezzanotte et al., 2013).

The BOD_5/COD (Table 1) showed that the original RWW, the RDB effluent, and the ozonated RWW, were non-biodegradable (<0.2) (Metcalf and Eddy, 2003). The only effluent that was found to be moderately biodegradable was that resulting from the treatment train. This is an important finding in favour of the treatment train since the effluents obtained would require a further biological treatment according to current regulations in Catalonia, such as a conventional activated sludge treatment, which could be performed *in-situ* or in a

Table 2
Pesticide and TPs contribution to the DOC during the ozonation treatment.

		Ozonation time (h)			
		0	4	8	12
Ozonation of UP water	Pesticides (mg C L^{-1})	59.9	0.0	0.0	–
	Oxalic acid (mg C L^{-1})	0.0	0.8	1.1	–
	Oxamic (mg C L^{-1})	0.0	0.6	0.9	–
	Maleic acid (mg C L^{-1})	0.0	0.0	0.0	–
Ozonation of RWW	DOC (mg C L^{-1})	1527	1399	1114	1073
	Pesticides (mg C L^{-1})	38.5	3.0	0.3	0.0
	Oxalic acid (mg C L^{-1})	[a]	[a]	[a]	[a]
	Oxamic (mg C L^{-1})	0.0	10.1	16.2	20.7
	Maleic acid (mg C L^{-1})	0.0	0.6	1.6	1.8
Bioremediation of RWW followed by ozonation	DOC (mg C L^{-1})	2981	2709	2700	2656
	Pesticides (mg C L^{-1})	19.1	5.0	0.5	0.0
	Oxalic acid (mg C L^{-1})	[a]	[a]	[a]	[a]
	Oxamic (mg C L^{-1})	0.0	3.3	5.8	8.2
	Maleic acid (mg C L^{-1})	0.0	0.0	0.7	0.5

Note: total DOC was obtained experimentally, while the DOC of oxamic, oxalic and maleic acid was calculated theoretically from their respective concentrations.
[a] Not determined due to interference with the matrix.

WWTP (DOGC, 2003).

Regarding the toxicity (Table 1), no major changes were observed. The increased toxicity in the ozonation of RWW was ascribed to the generation of some TPs, which can be even more toxic than the parent compounds (Cruz-Morató et al., 2013). Interestingly, a more remarkable decrease in toxicity was obtained in the case of the treatment train, suggesting some synergistic effect when combining both technologies.

Finally, the number of colony-forming units of total heterotrophs (at 37 °C) was also analysed (Table 1). Bacterial counts rose slightly after 67 days of bioremediation treatment while remaining below the LOQ in the ozonated effluents. Ozone has already proven to be a good strategy for bacterial removal (Gorito et al., 2021). In this case, no bacterial regrowth was observed even after keeping the samples in the refrigerator for several weeks.

3.7. Technology combination: Economic terms

3.7.1. Fungal bioremediation costs

The most significant bioremediation costs were related to electricity consumption. In this case, the HRT used in the RDB was considered as the calculation base, *i.e.*, 5 days, which is the time required to treat 2.7 L of RWW. Bioremediation costs include inlet pumping (BP), recirculation pumping (BR), air pumping (BA), magnetic stirring of recirculated RWW (BS), rotation of the inner tube (BRot), and preparation of the mycelial suspension (M), as shown in Eq. (1).

$$B = BP + BR + BA + BS + BRot + M \quad (1)$$

Each of these costs is in turn composed of the power of the equipment (W) multiplied by the operating time (t). Note that laboratory-scale devices were used, which are generally less efficient than industrial equipment. In the case of inlet pumping consumption, the pump has a maximum flow rate of 0.60 L h^{-1} and the filling time was 4.50 h (2.7 L), thus:

$$BP = BP_W \cdot BP_t = 5\ (W) \cdot 4.50\ (h) = 22.50\ Wh \quad (2)$$

The recirculation flow rate was 4.7 L day^{-1}, so it can be considered that the pump, with a maximum flow rate of 0.6 L h^{-1} and a maximum power of 5 W, worked for 39.17 h during 5 days.

$$BR = BR_W \cdot BR_t = 5\ (W) \cdot 39.17\ (h) = 195.85\ Wh \quad (3)$$

Aeration was supplied from day 28 onwards, *i.e.*, for 39 days, by using a general pump that worked for several installations. However, to simplify calculations, a small 0.5 W positive displacement electric pump was assumed to be used.

$$BA = BA_W \cdot BA_t = 0.5\ (W) \cdot 69.85\ (h) = 34.93\ Wh \quad (4)$$

The agitation in the recirculation tank (BS) was active during the entire operating period, being its cost significant and thus considered in the calculations. However, agitation of the feed tank was performed punctually four times per day, hence it was considered negligible and was excluded from the cost estimation.

$$BS = BS_W \cdot BS_t = 2.5\ (W) \cdot 120\ (h) = 300\ Wh \quad (5)$$

The power consumption resulting from the rotation of the internal drum was obtained as shown in Eq. (6).

$$BRot = BRot_W \cdot BRot_t = 19.20\ (W) \cdot 0.014\ (h) = 0.27\ Wh \quad (6)$$

Mycelial suspension preparation requires autoclaving (MA) and orbital shaking (MOS) steps (Section 2.1). Malt extract is an expensive reagent, but it can be perfectly substituted by a defined medium, as demonstrated in another report (Borràs et al., 2008). Other costs, such as the purchase of *T. versicolor* culture, were considered negligible.

$$M = MA + MOS \quad (7)$$

It can be reasonably assumed that preparation of the mycelium suspension can be performed every 6 months, as the RDB has been shown to require low biomass renewal (Beltrán-Flores et al., 2022). Therefore, these costs should be distributed over 6 months, and then the cost related to a period of 5 days should be computed. The autoclave operated for 2 h, whereas the orbital shaker worked for 5 days (120 h).

$$MA = MA_W \cdot MA_t = 2000\ (W) \cdot \frac{2\ (h) \cdot 5\ days}{182.5\ days} = 109.59\ Wh \quad (8)$$

$$MOS = MOS_W \cdot MOS_t = 30\ (W) \cdot \frac{120\ (h) \cdot 5\ days}{182.5\ days} = 98.63\ Wh \quad (9)$$

$$M = MA + MOS = 208.22\ Wh \quad (10)$$

Therefore, the bioremediation energy consumption accounted for one HRT unit (5 days) was as follows:

$$B = BP + BR + BA + BS + BRot + M$$
$$= 22.50 + 195.85 + 34.93 + 300 + 0.27 + 208.22 = 761.77\ Wh \quad (11)$$

Given that the average price of electricity in Spain is 0.28374 € $(kWh)^{-1}$ (July 2022) the cost of bioremediation for each 2.7 L of treated water (5 days) was calculated as shown in Eq. (12).

$$B = 761.77\ (Wh) \cdot 0.28374\ € \ (kWh)^{-1} = 0.22\ € \quad (12)$$

Therefore, the cost per cubic metre treated by fungal bioremediation is 81.48 € m^{-3}, as shown in Eq. (13).

$$B = \frac{0.22\ (€)}{2.7\ (L) \cdot 10^{-3} \left(\frac{m^3}{L}\right)} = 81.48\ € \ m^{-3} \quad (13)$$

The economic analysis focused on the ozonation period for both direct ozonation and the treatment train, thus the bioremediation cost of the treatment train was computed as a fixed cost (independent of the

ozonation time) prior to the ozonation stage (Sections 3.7.3 and 3.7.4).

3.7.2. Ozonation costs

The economic study focused on the ozonation period, thus time-dependent energy consumption and related costs were treated as function of ozonation time. Ozonation costs (O) can be separated into costs related to energy consumption (OE) and oxygen supply (OO).

$$O = OE + OO \tag{14}$$

The electrical costs of ozonation consisted of the consumptions of the pump (OP), the ozone generator (OG) and the magnetic stirrer (OS):

$$OE = OP + OG + OS \tag{15}$$

In turn, each of these costs was calculated based on power consumption (W) and working time (t). OP was considered a cost that was incurred during reactor filling prior to the beginning of treatment, and thus was independent of the operating time. OP_t was calculated as the division of the reactor volume (700 mL) by the maximum pump flow rate (600 mL h^{-1}).

$$OP = OP_w \cdot OP_t = 5\ (W) \cdot 1.17\ (h) = 5.85\ Wh \tag{16}$$

$$OG = OG_w \cdot OG_t = 80\ (W) \cdot OG_t(h) = 80\ OG_t\ Wh \tag{17}$$

$$OS = OS_w \cdot OS_t = 2.5\ (W) \cdot OS_t(h) = 2.5\ OS_t\ Wh \tag{18}$$

Given that OG_t and OS_t are equal to the ozonation time (O_t), and knowing the average price of electricity in Spain, the electrical cost of ozonation can be expressed as follows:

$$OE = OP + OG + OS = 1.65 \cdot 10^{-3} + 2.27 \cdot 10^{-2} OG_t + 7.09 \cdot 10^{-4}\ OS_t = \left(1.65 \cdot 10^{-3} + 2.34 \cdot 10^{-2} O_t\right)\ € \tag{19}$$

The oxygen cost (OO) was calculated by multiplying the oxygen flow rate (OO_Q) by the treatment time (O_t) and by the specific cost of oxygen (OO_c), as shown in Eq. (20). Oxygen consumption was 150 mL min^{-1} (9 L h^{-1}) and the price of oxygen was 50.20 € for each 10.6 m^3 bottle (0.00474 € L^{-1}).

$$OO = OO_Q \cdot O_t \cdot OO_c = 9\ \left(L\ h^{-1}\right) \cdot O_t(h) \cdot 0.00474\ \left(€\ L^{-1}\right) = 4.27 \cdot 10^{-2}\ O_t\ € \tag{20}$$

Therefore, the total cost of ozonation was:

$$O\ (1 - batch) = OE + OO = 1.65 \cdot 10^{-3} + 2.34 \cdot 10^{-2} O_t + 4.27 \cdot 10^{-2}\ O_t = \left[1.65 \cdot 10^{-3} + 6.61 \cdot 10^{-2}\ O_t(h)\right]\ € \tag{21}$$

This cost was relative to each ozonation batch, in which 0.7 L of RWW was treated. However, the same calculation basis of 2.7 L was used to compare the ozonation and bioremediation processes. For this purpose, the ozonation cost was multiplied by a factor of 3.86.

$$O = \left[6.38 \cdot 10^{-3} + 2.55 \cdot 10^{-1}\ O_t(h)\right]\ € \tag{22}$$

Changing O_t units from hours to minutes:

$$O = \left[6.38 \cdot 10^{-3} + 4.23 \cdot 10^{-3}\ O_t(min)\right]\ € \tag{23}$$

Therefore, the cost per cubic metre treated by ozonation can be calculated using Eq. (24).

$$O = \frac{\left[6.38 \cdot 10^{-3} + 4.23 \cdot 10^{-3}\ O_t(min)\right]\ (€)}{2.7\ (L) \cdot 10^{-3}\left(\frac{m^3}{L}\right)} = \left[2.36 + 1.57\ O_t(min)\right]\ € \ m^{-3} \tag{24}$$

3.7.3. Treatment train costs

Treatment train costs (T) were calculated by adding the costs of fungal bioremediation [Eq. (12)] and ozonation [Eq. (23)], as shown in Eq. (25).

$$T = B + O = 0.22 + \left[6.38 \cdot 10^{-3} + 4.23 \cdot 10^{-3}\ O_t(min)\right] = \left[0.23 + 4.23 \cdot 10^{-3}\ O_t(min)\right]\ € \tag{25}$$

Therefore, the cost per cubic metre treated by the treatment train can be calculated using the Eq. (26).

$$T = \frac{\left[0.23 + 4.23 \cdot 10^{-3}\ O_t(min)\right]\ (€)}{2.7\ (L) \cdot 10^{-3}\left(\frac{m^3}{L}\right)} = \left[85.19 + 1.57\ O_t(min)\right]\ € \ m^{-3} \tag{26}$$

3.7.4. Comparative study

The costs per cubic metre treated by the ozonation and treatment train previously calculated in Eq. (24) and Eq. (26), respectively, were used to obtain Fig. 6(a). As can be seen, the analysis focuses on the ozonation period, so other costs before this stage were considered as initial fixed costs. In this regard, the ozonation costs start from practically zero, since only the cost of filling the stirred tank was considered and was computed before the ozonation period. In the case of the treatment train, the bioremediation cost (in addition to the cost of filling the stirred tank in the ozonation stage) was considered fixed and computed at the beginning of the ozonation treatment [Eq. (26)], as shown in Fig. 6(a). The slopes of both equations [Eqs. (24) and (26)], and thus those of their respective representations in Fig. 6(a), are equal since they correspond to the ozonation cost per cubic metre of treated wastewater.

To simplify calculations, pesticide removal curves of Fig. 4 were considered to follow a linear trend during the first 180 min of ozonation, obtaining $R^2 = 0.9972$ for the ozonation process [Eq. (27)] and $R^2 = 0.9964$ for the treatment train [Eq. (28)]. The units of these linear equations of DOC elimination were transformed from % to mg C, obtaining the equations Eq. (29) for the ozonation treatment and Eq. (30) for the treatment train. Afterwards, the specific costs [Fig. 6(b)] of the direct ozonation of RWW were obtained by dividing Eq. (23) and Eq. (29), and the specific costs of the treatment train were calculated by dividing Eq. (25) and Eq. (30).

$$Removal\ of\ pesticide\ DOC\ by\ ozonation = 0.4711 \cdot O_t(min)\ (\%) \tag{27}$$

$$Removal\ of\ pesticide\ DOC\ by\ the\ treatment\ train = 0.1621 \cdot O_t(min) + 50.43\ (\%) \tag{28}$$

$$Removal\ of\ pesticide\ DOC\ by\ ozonation = 0.4893 \cdot O_t(min)\ (\text{mg C}) \tag{29}$$

$$Removal\ of\ pesticide\ DOC\ by\ the\ treatment\ train = 0.1643 \cdot O_t(min) + 52.81\ (\text{mg C}) \tag{30}$$

The specific costs of ozonation are initially high because the cost of filling the reactor (OP) was considered a fixed cost and was computed at time 0 when pesticide elimination was non-existent. However, as the reaction progressed, the direct ozonation process seemed to be a more cost-effective treatment than the treatment train for removing pesticides. This result is probably explained by the fact that during the bioremediation process, part of the organic matter of the wood was extracted, increasing the DOC of the water, which also interacts with ozone molecules and the generated radicals. According to Fig. 6(b), the specific cost of ozonation was higher during the first 84 min, but then it was exceeded by the specific costs of the treatment train.

Another way of expressing these results that can further facilitate the selection between the different approaches is the correlation between the costs per cubic metre [Eqs. (24) and (26)] and the percentage of pesticides eliminated [Eqs. (27) and (28)], as shown in Fig. 6(c). For the same pesticide removal yield (≈50 %), the fungal bioremediation (85.19 € m^{-3}) proved to be 2.00 times cheaper than the ozonation treatment (170.42 € m^{-3}, Fig. 6(c)). However, bioremediation was only able to remove 50 % (19.40 mg C L^{-1}) of the pesticides (38.47 mg C L^{-1} in the RWW, Table 2). Ozonation was required to achieve higher removals,

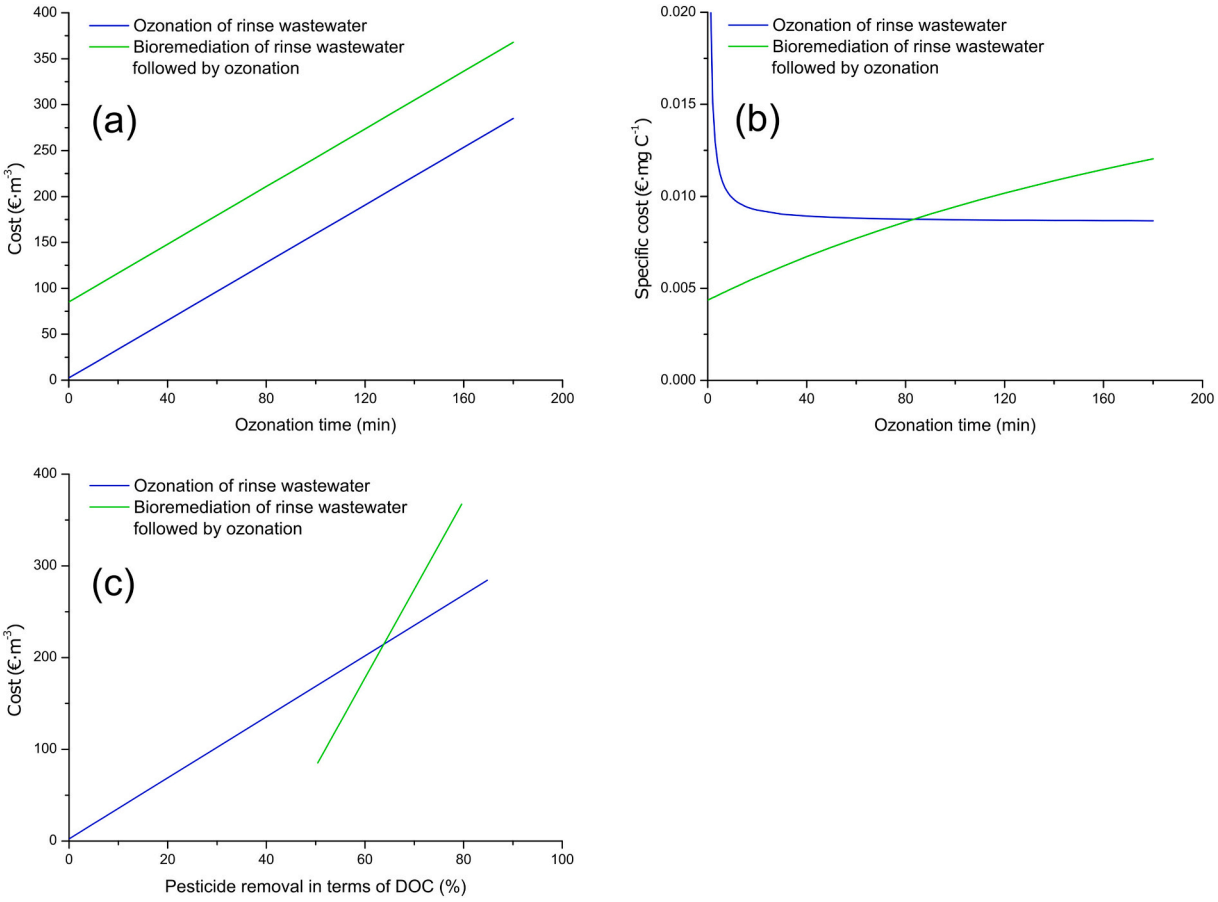

Fig. 6. Analysis of operating costs: evolution of costs per cubic metre during the ozonation stage (a), evolution of specific costs per mg of C removed during the ozonation stage (b) and correlation between costs per cubic metre and pesticide removals in terms of DOC (c).

either directly applied or as the second stage in the treatment train. Fig. 6(c) indicates that the cost associated with the application of the treatment train was lower than that of direct ozonation for eliminations below 64 %. Therefore, these results show that the selection of any of these technologies in economic terms depends on the requirements for effluent quality. If the target pesticides are supposed to be completely eliminated, direct ozonation is mandatory. In contrast, if the requirements for pesticide concentrations in the effluent are more flexible, which is often dictated by the current regulation, a treatment train (for eliminations between 50 and 64 %) or a bioremediation process (for elimination lower than 50 %) should be applied. However, it should be considered that the results of other studied parameters can also influence the selection of the most appropriate technology, such as effluent biodegradability and formation of TPs (Sections 3.5 and 3.6).

4. Conclusions

Fungal bioremediation achieved an average removal of the studied pesticides equivalent to 50 % in terms of DOC in a continuous RDB after 67 days (HRT = 5 days). The ozonation treatment proved to be more effective in removing pesticides, achieving complete removal after 4 h in ultrapure water, and after 12 h in RWW and RDB effluent (treatment train). Bioremediation, unlike ozonation, did not produce any of the studied TPs and even reduced their generation by ozonation when applied in an upstream stage in the treatment train. Concerning other conventional parameters, ozonation was more effective in reducing COD (31 %), DOC (30 %), and HPCs (100 %). Furthermore, the only effluent found to be moderately biodegradable was that from the treatment train, with a BOD_5/COD ratio of 0.30. The cost study revealed that the fungal bioremediation treatment was approximately 2 times more cost-effective (85 € m^{-3}) compared to ozonation (170 € m^{-3}). Based on the economic cost study, fungal bioremediation is recommended for pesticide removals below 50 %, the treatment train for removals between 50 and 64 %, and direct ozonation for removals above 64 %. Therefore, the treatment train proved to be an interesting approach that integrates the advantages of both processes, thus offering more flexibility to the relationship between effluent quality and operating costs.

CRedit authorship contribution statement

All authors have approved the final version of the manuscript. All authors have materially participated in the research and/or preparation of the article, as follows:

Eduardo Beltrán Flores: conceptualization, data curation, formal analysis, investigation, methodology, writing original draft; **Paqui Blánquez**: conceptualization, funding acquisition, project administration, resources, supervision, validation, writing review & editing; **Ana M. Gorito**: investigation, methodology, writing review & editing; **Montserrat Sarrà**: conceptualization, funding acquisition, project administration, resources, supervision, validation, writing review & editing; **Adrián M. T Silva**: conceptualization, funding acquisition, project administration, resources, supervision, validation, writing review & editing.

CRediT authorship contribution statement

Eduardo Beltrán-Flores: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Paqui**

Blánquez: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Ana M. Gorito:** Methodology, Writing – review & editing. **Montserrat Sarrà:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Adrián M.T. Silva:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169198.

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