Science of the Total Environment 972 (2025) 179029

Contents lists available at ScienceDirect

Science of the Total Environment

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

Life cycle assessment of UVC-based advanced oxidation processes as quaternary treatments: *Clostridium* spp. inactivation and comparison with CECs removal

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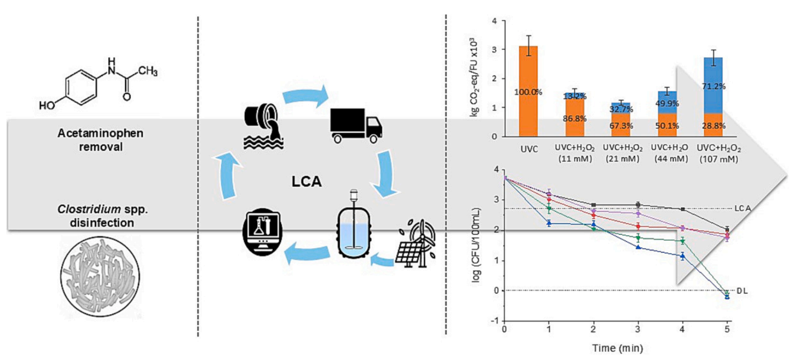
HIGHLIGHTS

- UVC/H_2O_2 process is the most effective method to remove *Clostridium* spp. from WWTP effluents.
- LCA shows that UVC photolysis was more sustainable, due to the lower chemicals consumption.
- Circumneutral photo-Fenton is more efficient for acetaminophen removal, also showing lower GWP.
- Chemical decontamination shows higher GWP than disinfection.
- LCA revealed laboratory experiments have lower impact, when compared with sample transportation and analysis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jacopo Bacenetti

Keywords:
Life cycle assessment (LCA)
UVC photolysis
UVC/peroxide
Photo-Fenton
Clostridium disinfection

ABSTRACT

In this work, Life Cycle Assessment has been applied for the evaluation of UVC-based photochemical treatments to deal with wastewater treatment plant effluents. In particular, UVC photolysis, UVC/H_2O_2 combination and $UVC/H_2O_2/Fe(II)$ (namely photo-Fenton) were tested at pH ca. 7.5. *Clostridium* spp. was chosen as target species to test disinfection. Both, UVC/H_2O_2 and photo-Fenton were able to decrease *Clostridium* spp. below detection limit in 5 min. Best results were reached with H_2O_2 concentration of 21 mM. Coupling coagulation-flocculation with the photochemical process was tested for effluents with high organic load and turbidity. Global warming potential selected UVC photolysis as the best treatment for disinfection from the environmental point of view, despite its lower efficiency. However, when CECs removal was considered using acetaminophen as target contaminant, photo-Fenton was the most efficient and the most sustainable option. Finally, LCA was applied to the experimental work performed in the laboratory, showing that the effect of reactions was negligible vs. sample

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https://doi.org/10.1016/j.scitotenv.2025.179029
Received 15 November 2024; Received in revised form 25 February 2025; Accepted 28 February 2025
Available online 15 March 2025

collection and transportation, as well as analytical procedures which accounted for more than 99 % of the impact.

1. Introduction

One of the major concerns for humanity is water scarcity. Population growth and related economic development have resulted in increasing water demand and consequent pressures on water availability (Liu et al., 2017). Therefore, the use of non-conventional water resources is required, and water reuse seems a promising approach for this purpose. (Abou-Shady et al., 2023). Recycling is expected to have a major impact on water management in the 21st century with potential uses in irrigation of crops, gardens or golf courses, selected industrial activities, as well as groundwater recharge (Miller, 2006). However, it should be noted that this water must satisfy certain conditions; for instance, in the European Union, treated effluents must fulfil the regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for water reuse; more examples on water reuse legal framework around the World can be found elsewhere (Pereira Santos et al., 2024).

Conventional wastewater treatment plants (WWTPs) are required to comply with the Urban Wastewater Treatment directive and Water Frame directive (Salgot and Folch, 2018), but they are not always efficient enough to reach the required levels of quality for reuse; in particular, the presence of microorganisms or xenobiotics at low concentrations represents a major limitation for reuse (Petrie et al., 2015; León et al., 2019; Kumar et al., 2022). Among xenobiotics, contaminants of emerging concern (CECs) can be remarked. They are commonly found at low concentrations (ng-μg/L) and their actual effect on the environment is not completely established. Some examples of CECs are pharmaceuticals, pesticides or personal care products (Jiang et al., 2013; Galindo-Miranda et al., 2019).

Regarding micro-organisms, *Clostridium* ssp. is one of the most common families of bacterial pathogens in water and it is an indicator of disinfection, because of its reluctance towards routine treatments (Rupnik et al., 2009). There are more than 200 different *Clostridium* species but the most prevalent ones that affect human health are *C. difficile, C. tetani, C. botulinum, C. perfringens,* and *C. septicum.* All are etiological agents of devastating diseases in both humans and animals, making them significant from a medical and veterinary perspective. (Songer, 2010; Sánchez-Sánchez et al., 2020). Thus, ensuring that efficient disinfection has been reached is needed before water can be reused (Weber and Rutala, 2011; Stevik et al., 2004).

Advanced oxidation processes (AOPs) are among the most promising methods to be implemented as quaternary treatments to refine water quality (Zhang et al., 2022a; Rizzo, 2022). Among AOPs, ultraviolet C (UVC) irradiation is commonly used in WWTP, and its effect can be enhanced by the addition of peroxides and eventually iron salts (Zhang et al., 2022b; Wacławek et al., 2017; Pignatello et al., 2006). UVC irradiation is capable of decomposing hydrogen peroxide (H_2O_2) into hydroxyl radicals (·OH), which are highly reactive species able to oxidize a wide range of chemicals (Lopez et al., 2000). Interestingly, iron salts catalyse the formation of ·OH, according to the photo-Fenton process (Pignatello et al., 2006).

However, it is challenging to determine the best process since each has its drawbacks. For example, UVC alone might require longer irradiation times, UVC combined with H_2O_2 involves the addition of an extra reagent, and (photo)-Fenton needs an acidic environment to operate under optimal conditions. In this context, Life Cycle Assessment (LCA) is a key tool to determine which is the best process from an environmental point of view. LCA can be determined according to standardized ISO 14040:2006 and ISO 14044:2006 (ISO, 2006a; ISO, 2006b) and it is a technique used to examine a product, process or system from an environmental perspective, by evaluating the influence of energy, reagents, water matrices and waste on different impact categories. LCA has been widely used to quantify environmental impacts associated with urban water infrastructure, including WWTPs (Byrne et al., 2017) as it captures trade-offs along different categories of environmental concern (i.e., carbon emissions, water consumption, emission of toxic species, etc.). It serves as a useful decision-making support tool to examine alternative scenarios of operation alongside and during strategic planning of the wastewater sector (Tsangas et al., 2023). Since many processes are involved and their effects are interdependent, the application of LCA to WWTP seems rather challenging (Corominas et al., 2020).

With this background, the aim of this work is to provide more information on the applicability of UVC-based treatments as a quaternary process, not only based on the efficiency but also including a LCA evaluation. For this purpose, data published in a previous article on acetaminophen removal by UVC, UVC/H_2O_2 and UVC-driven photo-Fenton at mild conditions will be employed (López-Timoner et al., 2023). Acetaminophen has been selected as a target pollutant since it is a widely used anti-inflammatory drug commonly found in the environment (Burns et al., 2018) and in WWTP effluents (Martínez Bueno et al., 2012). In addition, experiments on the ability of the processes for disinfection will be performed, using *Clostridium* ssp. as indicator. It has been selected because C. perfringes (spores) is one of the microbial indicators included in the validation monitoring program for any new or upgraded water reclamation facilities established in the current EU Regulation, 2020/741. Finally, the impact of each process will be assessed with the total potential burden in a common WWTP to quantify the contribution of the additional process to the whole wastewater treatment impacts.

2. Materials and methods

2.1. Reagents and reactions

Four different water matrices were used in this study (main characteristics can be seen in Table 1): (i) MilliQ® water, (ii) WWTP1, taken from the clarifier of the secondary treatment of the WWTP of Alcoy (Spain), (iii) WWTP2, taken from the same point of WWTP of Alcoy, but spiked with activated sludge in order to increase turbidity and organic loading and (iv) WWTP2c-f that consisted in WWTP2 submitted to a coagulation-flocculation process explained below.

Hydrogen peroxide (H_2O_2, 33 % *w/v*) was supplied by PanReac AppliChem, heptahydrated iron(II) sulfate ($FeSO_4·7H_2O$) and bovine catalase were purchased from Sigma Aldrich. Ferric sulfate was also used as a coagulant and Magnafloc® was the polyelectrolyte employed as flocculant.

Irradiations were carried out using a 15 W low-pressure mercury

Table 1
Summary of some key parameters of the water matrices used in this work.

Parameter	MilliQ® Water	WWTP1	WWTP2	WWTP2c-f
Dissolved organic carbon (DOC) (mg L^{-1})	0	12.3	134	16
Clostridium spp. (CFU/100 mL)	0	6.10×10^3	190×10^3	6.70×10^3
Conductivity (μS cm^{-1})	8	540	670	721
Turbidity (NTU)	0	11.2	567	3.2
pH	6.98	7.50	7.47	7.14

lamp (Heraeus Noblelight) emitting nearly monochromatic radiation at 254 nm, protected by a quartz envelope and placed axially in a closed glass reactor (8 cm diameter, 25 cm height). The system was refrigerated using an outer water jacket (see López-Timoner et al., 2023 for further details on the used set-up). The reactor was loaded with 0.5 L of the solution to be treated. In the experiments involving (H_2O_2), concentrations in a range of 0–88 mM were employed and they were added before irradiation. In the case of photo-Fenton, also Fe(II) was added to reach a concentration of 11 μM (0,62 mg L^{-1}); this low concentration was chosen to accomplish with regulations (e.g. 5 mg L^{-1} iron Valencia Regional Government) (Entidad de Sanejament d'Aigües (EPSAR). Ordenanza de vertidos. València, España, 2024). Experiments were also carried out without irradiation (dark controls).

In some experiments with WWTP2, samples were treated with and without a previous coagulation-flocculation process. For this operation, a jar-test was performed following the standard procedure (ASTM Standards, 1986). 10 mL of the ferric sulfate solution (10 g L^{-1}), used as coagulant, was added to 1 L of sample, and the mixture was stirred for 1 min at 120 rpm. Then, 5 mL of the Magnafloc® flocculant (0.3 mg L^{-1}) was added and stirred at 30 rpm for 5 min. Finally, the solution was kept for 1 h in an Imhoff cone, in order to allow the solids to settle down, and the supernatant was recovered to be submitted to photochemical treatment.

2.2. Bacterial strains enumeration and quantification

For *Clostridium* ssp. analysis, 250 mL of solution were taken from the reactor after the desired irradiation time (0–5 min). Then, 5 mL of bovine catalase (0.1 g L^{-1}, prepared from a solid product supplied by Sigma Aldrich) were added to the samples to remove the remaining H_2O_2 (Bianco et al., 2017).

The enumeration and quantification of naturally occurring spores of *Clostridium* spp. were made following the standardized procedure (ISO 14189:, 2017). Briefly, 100 mL of each sample were filtered through membrane filters (sterile cellulose nitrate, 47 mm, 0.45 μm) by using a vacuum pump. When a high number of colonies was suspected, solutions were previously diluted with demineralized water (ISO 8199:, 2018).

Filters were placed in a TSC Cycloserine Bioagar medium by Akralab. Then, samples were incubated under anaerobic conditions for 21 ± 3 h at 44 ± 1 °C and colonies were counted. Anaerobic conditions were achieved by placing the corresponding filters upside down in a special bag with a reagent that provides a nitrogen atmosphere. The detection limit (DL) was 1 CFU/100 mL.

2.3. Life cycle assessment (LCA)

LCA is a strategic technique used to identify and quantify the potential environmental impacts associated with a product, process or system throughout its life cycle, according to the international ISO standards 14,040:2006/Amd 1:2020 and 14,044:2006/Amd 1:2017 + Amd2:2020 (ISO, 2006a, 2006b). The common LCA framework applies well-regarded scientific mechanisms and characterization models and consists of 4 main phases: i) Goal and scope definition; ii) Life Cycle Inventory (LCI); iii) Life Cycle Impact Assessment (LCIA); and iv) Interpretation). These four phases are described below with reference to the system under investigation.

2.3.1. Goal and scope definition

The aim of the study is to estimate the environmental impacts associated with the amount of energy and reagents required to *i)* decrease the concentration of *Clostridium* spp. by one order of magnitude in 0.5 L of inlet water and *ii)* with the amount of energy and reagents required to decrease the acetaminophen concentration from an initial value of 1000 μg/L to fall below the detection limit (ca. 50 μg/L). The treatments are designed for laboratory scale and are meant to reduce or eliminate the presence of organic and bacteriological contaminants naturally occurring in the water exiting from WWTPs. Processes are summarized as follows: (i) UVC irradiation, (ii) UVC + H_2O_2, and (iii) photo-Fenton process (UVC + H_2O_2 + Fe(II)). More details about the conditions were reported in section 2.1.

The water discharged from the wastewater treatment system is already compliant with legislative requirements. This study aims to assess the effectiveness of additional processes designed to further reduce the presence of contaminants that, while within legal limits, can still be minimized, and to estimate the related potential environmental impacts. For this reason, the amount of energy and reagents required to decrease the concentration of *Clostridium* spp. by one order of magnitude in 0.5 L of inlet water for disinfection was selected as a functional unit (FU), to provide a reliable comparison among the treatments. The choice depended on the capacity of the reactor used for the experiments. For the removal of CECs, the FU was the amount of energy and reagents employed to decrease the acetaminophen concentration from an initial value of 1000 μg/L to fall below the detection limit (ca. 50 μg/L).

As said, LCA is applied to a system designed for the lab scale. For this reason, it must be seen as a preliminary approach to identify the potential hotspots of a future facility. However, it should be noted that the impacts associated with laboratory-scale systems are -in general- higher compared to industrial plants. This is due to the configuration of the apparatus that is then optimized at higher scale, both in terms of material and energy flows. The optimization allows the mitigation of the impacts, dispersed over larger volumes.

The system boundaries of the evaluation include, in addition to the treatments, the coagulation-flocculation process (when necessary) and the analysis of the samples. This is a *cradle-to-gate* approach. Specifically, the LCA model includes the upstream processes related to chemicals, energy, and auxiliaries employed in the core phase, i.e., the treatment process for cleaning water. A zero-burden criterion is applied to the inlet water since it is considered a waste without economic value (Lam and Hoek, 2020). Since the infrastructural components were the same for all the scenarios evaluated, they were excluded from the model by applying an equivalence cut-off (ISO, 2006b). A hotspot analysis was also performed to check the contribution of each process in the system. To obtain a more accurate comparison among processes, three different calculations were performed: (scenario 1) including all the processes, and (scenario 2) considering only the AOP. Scenario 2 is modelled by assuming that, at an industrial scale, the analysis of the discharged water is expected to be conducted periodically (either time-based or volume-based) and should not significantly affect the overall impacts. Same for transportation, since this phase could be avoided if the additional treatment plant is placed in proximity of the WWTP. Mass and energy flow diagrams for *Clostridium* ssp. disinfection and acetaminophen removal are shown in Fig. 1a and b. No allocation criteria were applied in the study.

2.3.2. Life cycle inventory (LCI)

The inventory of the acetaminophen removal system was modelled according to the primary data, constituted by reagents and electricity used in previous work (López-Timoner et al., 2023) and, in the case of *Clostridium* ssp. disinfection, from a new experimental campaign. Some secondary data were obtained based on the relevant literature or from the ecoinvent 3.9 database (Wernet et al., 2016). The electricity flow was modelled according to the last available data from the International Energy Agency (IEA) related to Spain (IEA, 2023). Reagents, materials, equipment and energetic flows involved in each step of the system were reported in Table 2. The product system was modelled on SimaPro 9.5 (PRé Consultants, 2022).

2.3.3. Life cycle impact assessment (LCIA)

Results are reported according to the ReCiPe 2016 Life Cycle Impact Assessment (LCIA) method v. 1.08, perspective (H) (Huijbregts et al., 2017). The hierarchical perspective was set as the default perspective since it is considered the most representative of the geographical context

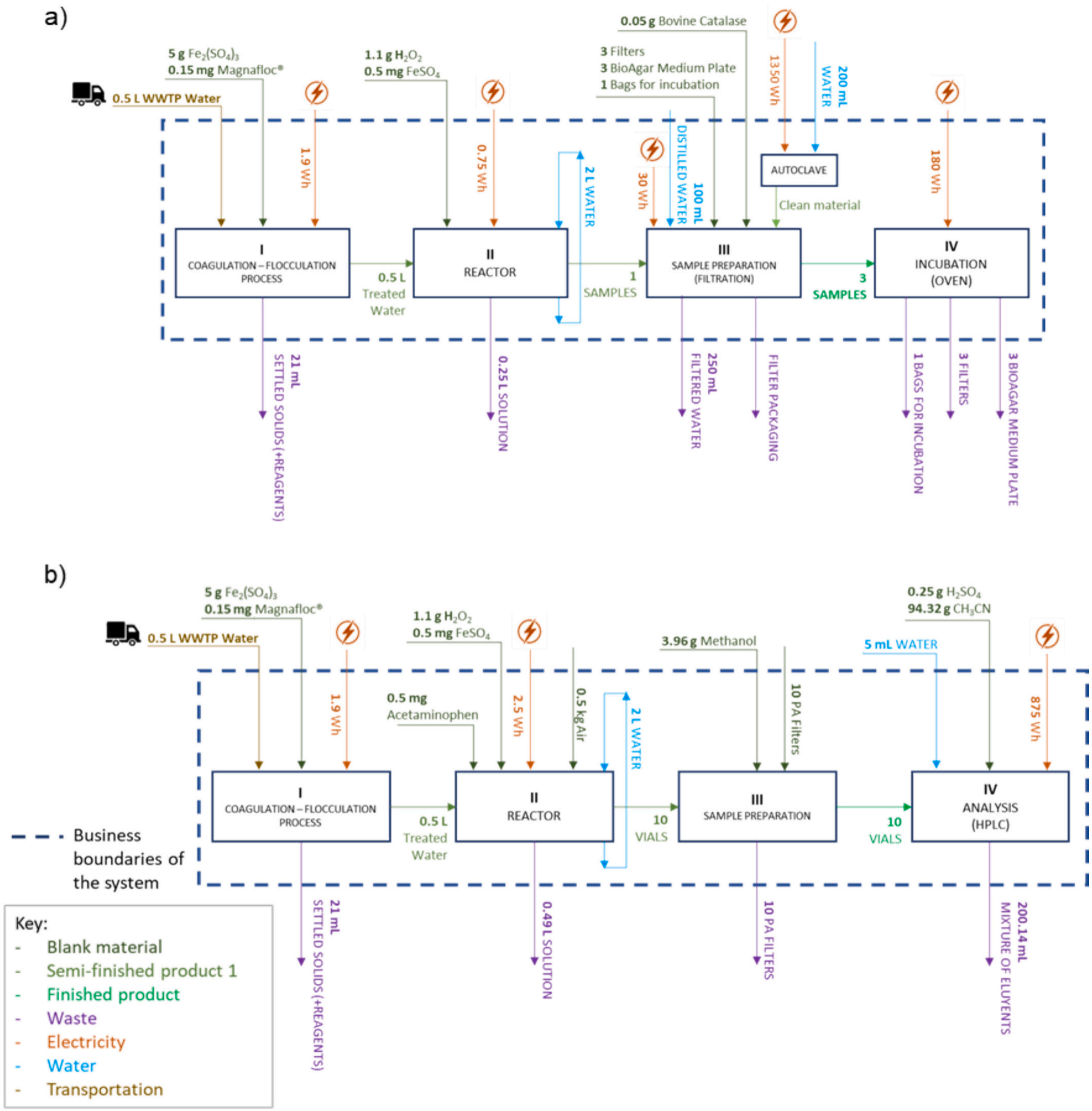

Fig. 1. Mass and energy flow diagram of a) *Clostridium* spp. disinfection and b) acetaminophen degradation process.

object of study. ReCiPe 2016 provides a comprehensive estimation of the interactions between the system under scrutiny and the environment for a set of 18 midpoint categories, namely: Global Warming Potential (GWP); Stratospheric Ozone Depletion Potential (ODP); Ionizing Radiation Potential (IRP); Ozone formation Potential-Human health (HOFP); Fine Particulate Matter Formation Potential (PMFP); Ozone Formation Terrestrial Potential-Ecosystems (EOFP); Terrestrial Acidification Potential (TAP); Freshwater Eutrophication Potential (FEP); Marine Eutrophication Potential (MEP); Terrestrial Ecotoxicity Potential (TETP); Freshwater Ecotoxicity; Potential (FETP); Marine Ecotoxicity Potential (METP); Human Carcinogenic Toxicity (HTPc); Human non-Carcinogenic Toxicity (HTPnc); Land Occupation Potential (LOP); Mineral Resource Scarcity (SOP); Fossil Resource Scarcity (FFP); Water Consumption Potential (WCP). The choice is principally motivated by the high diffusion of the method in the relevant literature related to the wastewater sector (Deeney et al., 2023; Lamnatou et al., 2022; Miniakhmetova et al., 2022).

2.3.4. Sensitivity and uncertainty analysis

The outcomes of the contribution analysis were taken as a reference to elaborate the sensitivity analysis, performed to test the robustness of the model created and enable identification and quantification of the influence of the main exogenous parameters onto the environmental impact of the entire system (Goedkoop, 2020).

Uncertainty evaluation was performed at midpoint level. In general, as discussed above, the LCA model for the system under scrutiny was

Table 2
Data inventory of the processes involved in the degradation of micropollutants since the transportation of the wastewater from the WWTP to the final analysis in the laboratory.

Macro flow	Type of flow	Amount and unit	Climate Change (kg CO_2-eq)	Data source
Transportation	Car from WWTP	11 km	0.23	Direct measure
Energy	Jar Test	1.9 Wh	0.21	(Velp Scientifica)
	UVC Lamp	3.75 Wh	0.21	(Heraeus Noblelight)
	Pump 1 (Scale-up)	5 Wh	0.21	(Resun)
	Pump 2 (Air)	3 Wh	0.21	(Resun)
	HPLC	875 Wh	0.21	(Hitachi Chromaster)
	Oven	180 Wh	0.21	(Nahita)
	Autoclave	1.35 kWh	0.21	(JP Selecta)
	Vacum pump	30 Wh	0.21	(Greiffenberger GmbH)
Materials	Distilled water	0.5 L	2.6×10^{-3}	(Milli-Q®)
	Ferric sulfate	5 g	–	(Sigma Aldrich)
	Magnafloc®	0.15 mg	0.11	(Sigma Aldrich)
	Hydrogen peroxide	1.10 g	1.06	(PanReac AppliChem)
	Iron (II) sulfate	0.50 mg	0.20	(Sigma Aldrich)
	Acetaminophen	1 mg	–	(Sigma Aldrich)
	Methanol	3.96 g	0.63	(PanReac AppliChem)
	Sulfuric acid	0.25 g	0.09	(PanReac AppliChem)
	Acetonitrile	94.32 g	4.73	(PanReac AppliChem)
	Polyamide filter	27 g	5.21	(Macherey-Nagel)
	Bovine catalase	0.05 g	–	(Sigma Aldrich)
	Membrane filters	0.20 g	0.38	(Macherey-Nagel)
	Bags for incubation	8.76 g	2.38	(BD GasPak TM EZ)
	TSC Cycloserine	43.50 g	0.09	(Bioagar)

entered with primary data obtained by laboratory experiments. As such, input data can be considered very reliable and fulfil the highest scores for data quality criteria commonly applied in LCA such as, for instance, geographical, temporal, and technological representativeness. In general, the pedigree matrix was taken as a reference for the uncertainty assigned (Weidema and Wesnæs, 1996). More details on the Monte Carlo results are reported as Supplementary Materials (Table S2, S4, S6 and S8).

3. Results and discussion

3.1. *Clostridium spp. inactivation by UVC radiation*

Fig. 2 shows the inactivation of *Clostridium* spp. in WWTP1 by different treatments based on UVC, and dark controls with and without H_2O_2. In the dark without H_2O_2, the concentration of *Clostridium* spp. remained constant at 5.5×10^3 CFU/100 mL for 5 min of treatment time (data not shown). When H_2O_2 was present in the dark (21 mM), a slight reduction of the concentration was observed in 5 min (0.9 Log Reduction Value (LRV)). In sharp contrast, a 1.7 LRV was reached under UVC irradiation without H_2O_2, while the UVC/H_2O_2 process (21 mM) and UVC-driven photo-Fenton process (11 μM of Fe(II), 21 mM H_2O_2 at neutral pH) were able to reach *Clostridium* ssp. concentration below the limit of detection (LD), namely 1 CFU/100 mL at 5 min of treatment time. As very similar results were reached by UVC/H_2O_2 and neutral UVC-driven photo-Fenton, it seems that the addition of iron is not necessary in this case, as most of the effect is attributable to the

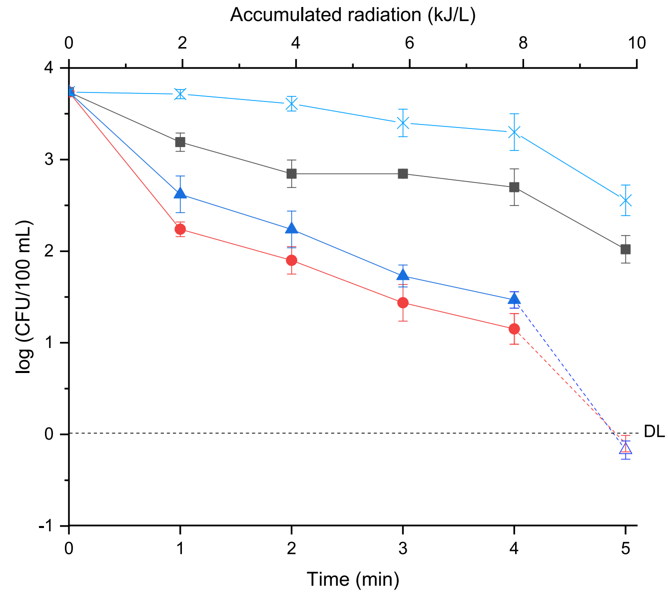

Fig. 2. Logarithmic plot of *Clostridium* ssp. (log(CFU/100 mL)) vs. time and accumulated radiation under different conditions (CFU_0/100 mL = 6.10×10^3; pH_0 = 7.5) when using WWTP1: 21 mM of H_2O_2 in the dark (X), UVC irradiation (■), UVC + 21 mM of H_2O_2 (▲) and UVC + 21 mM of H_2O_2 + 11 μM of Fe (II) (●).

synergetic effect of UVC and H_2O_2, which results in the formation of highly reactive hydroxyl radicals (·OH) (Lopez et al., 2000). These results agree with the literature, where under specific operational conditions, photo-Fenton at near neutral medium shows similar or even worse performance than solar H_2O_2 alone for water disinfection. This is attributed to the fact that photo-Fenton has been demonstrated to be efficient for disinfection only when it is conducted at the optimum pH conditions (2.8); while above this value, iron inactivation reduces the catalytic performance and even using photoactive iron chelates, disinfection is significantly inhibited (Rodríguez-Chueca et al., 2014; García-Fernández et al., 2019); This explains the results of *Clostridium* spp. as UVC treatments have been carried out at neutral pH.

The concentration of H_2O_2 was tuned in the range of 0 to 88 mM, in a series of experiments carried out under UVC irradiation and using WWTP1. Fig. 3 shows that an increase in the concentration of H_2O_2 resulted in an enhanced efficiency until reaching 44 mM and then, a very significant loss of efficiency was observed for 88 mM of H_2O_2. In fact, only for the experiments carried out with 21 mM and 44 mM of H_2O_2, the detection limit of *Clostridium* ssp. (1 CFU/100 mL) was reached. This is a well-known behaviour for H_2O_2-based treatments: at low concentrations, the addition of more peroxide results in an improved generation of ·OH; however, at higher H_2O_2 concentration, recombination of the formed ·OH or reaction of this radical with H_2O_2 is favored, thus decreasing the available amount of reactive species (Lopez et al., 2000).

A previous coagulation-flocculation treatment might be helpful for water showing high turbidity and organic loading, as recently shown for UVC-based photochemical processes using the same experimental system (López-Timoner et al., 2023). To obtain initial samples with these characteristics, WWTP was spiked with activated sludge, as indicated in the experimental section, to obtain WWTP2. After submitting this sample to the coagulation-flocculation process, WWTP2c-f was obtained, with the characteristics indicated in Table 1. It can be observed that the process was very efficient in treating turbidity and dissolved organic carbon, but also in decreasing the concentration of *Clostridium* spp., which dropped from 190×10^3 CFU/100 mL to 6.7×10^3 CFU/100 mL.

Fig. 4 shows the *Clostridium* spp. inactivation following both strategies: i) coagulation-flocculation to obtain WWTP2c-f followed by UVC/

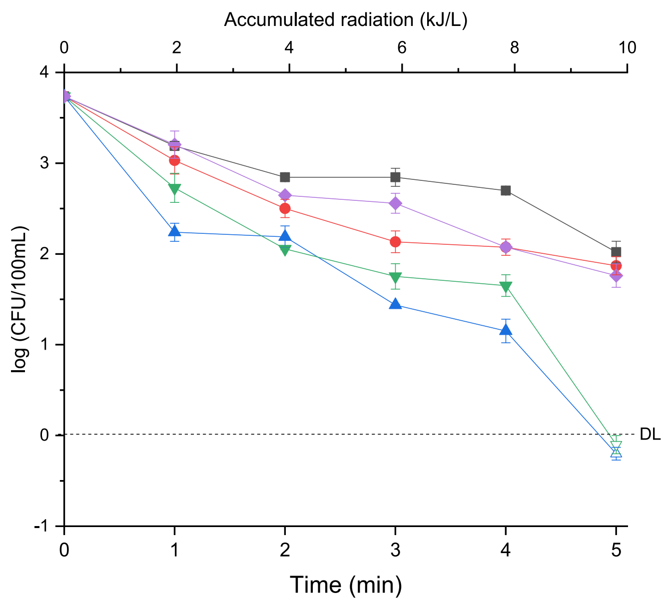

Fig. 3. Logarithmic plot of *Clostridium* ssp. (log(CFU/100 mL)) vs. time and accumulated radiation under different concentrations of hydrogen peroxide (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) when using WWTP1: UVC irradiation (■), UVC + 11 mM of H_2O_2 (●), UVC + 21 mM of H_2O_2 (▲), UVC + 44 mM of H_2O_2 (▼) and UVC + 88 mM of H_2O_2 (♦).

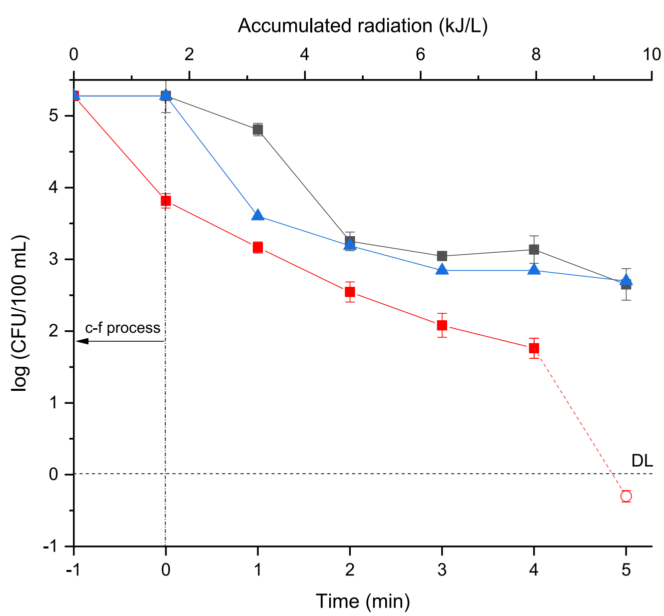

Fig. 4. Logarithmic plot of *Clostridium* ssp. (log(CFU/100 mL)) vs. time and accumulated radiation in the presence of 21 mM of H_2O_2 in different water matrices: WWTP1 (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) (▲), WWTP2 (CFU_0/100 mL = 190 × 10^3; pH_0 = 7.47) (■) and c-fWWTP2 (CFU_0/100 mL = 6.70 × 10^3; pH_0 = 7.14) (●).

H_2O_2 (44 mM), and ii) direct UVC/H_2O_2 treatment of WWTP2. While in the first strategy, *Clostridium* ssp. was below DL at $t = 5$ min, in the second case significant concentration of *Clostridium* ssp. was still detected at the same treatment time (448 CFU/100 mL), and thus, complete decontamination could not be achieved.

3.2. LCA of the disinfection processes

3.2.1. Comparison among treatments based on LCA

The comparison of the environmental performances is provided only for the reactor: UVC, UVC + H_2O_2 (21 mM) and the photo-Fenton process (UVC + H_2O_2 (21 mM) + Fe (11 μM)) (Fig. 5a). Fig. 5a shows the GWP results referred to 5 min of each process. If the same irradiation time is considered for all three cases, the impact associated with electricity consumption will be equal for all the alternatives and the difference between the performances will reflect only the amount of the employed reagents, especially H_2O_2. From the outcomes, it emerges that assuming to apply the three processes for 5 min, UVC represents the most sustainable treatment, although it does not allow complete disinfection. Conversely, for UVC + H_2O_2 and photo-Fenton, 5 min are sufficient to achieve a concentration within the legal limits. Assuming to operate exclusively in the presence of UVC light to perform the treatment, the GWP of the photolytic process would equate to the GWP of the other two treatments (UCV-photo-Fenton at UVC-peroxide) after 12.3 min. This means that, once this period is exceeded, the process using only UV light would become more carbon-intensive.

A more accurate comparison of the three treatments can be obtained when GWP was calculated for the decrease in one logarithmic unit of the initial concentration of *Clostridium* spp. (Fig. 5b). It can be observed there that under those circumstances, UVC irradiation is still more sustainable than the other treatments, as the effect of reagent addition was demonstrated to be dominant.

According to ISO 14044, the analysis was extended to all the environmental categories available in the ReCiPe 2016 LCIA method. The whole set of results is reported as Supplementary Material (Tables S1), while Fig. 6 depicts the results referred to METP (a), PMFP (b), HTPc (c), HTPnc (d), LOP (e) and FFP (f), since they demonstrated to be the ones that mainly contribute to the ReCiPe 2016 single score, confirming what it is observed by Arfelli and colleagues (Arfelli et al., 2022). Regarding the other environmental categories investigated, the best scores have been reached by UVC (green) according to the chosen FU, again because of the absence of further reagents. The uncertainty analysis, reported in Table S2, does not allow to assess a net preference between Photo-Fenton and UVC + H_2O_2. However, concerning GWP, PMFP, LOP and FFP, this issue is not attributable to the data quality, but to the similarity of the values obtained for the two processes. In the case of HTPc and HTPnc, the uncertainty ranges do not attribute a net preference between the three alternatives at a confidence level of 95 %.

The high standard deviation associated with HTc and HTnc represents a limitation in the estimation of the results since it does not allow to state a preference among the alternatives with certainty. The uncertainty may depend both on the inventory data quality and the selected LCIA method. In this case study, data is mainly derived from primary information, reflecting their high quality. Due to this, HTc and HTnc values could be justified by the intrinsic uncertainty associated with the toxicity evaluation in the ReCiPe 2016 method, as already observed in previous studies (Chen et al., 2021; Rossi et al., 2024). Specifically, the cause-effect model associated with the toxicity effects depends on several factors such as chemical-physical characteristics of the substance, mobility and transportation inside and between the environmental compartments, persistence, exposure models and interactions with sensitive targets, the response of the sensitive target (Hauschild et al., 2018). The high number of factors affecting the final toxic effect involves the existence of estimated values. Furthermore, the high uncertainty underscores the need to develop regionalized models (Mutel et al., 2019; Pfister et al., 2020) particularly when it is known, as in this case, that certain emissions occur in specific geographical areas. This is especially relevant for impact categories that have localized effects rather than global ones (i.e., PMF, HTc, HTcn and LO).

3.2.2. Effect of the addition of hydrogen peroxide

Regarding the effect of H_2O_2 concentration on GWP impacts

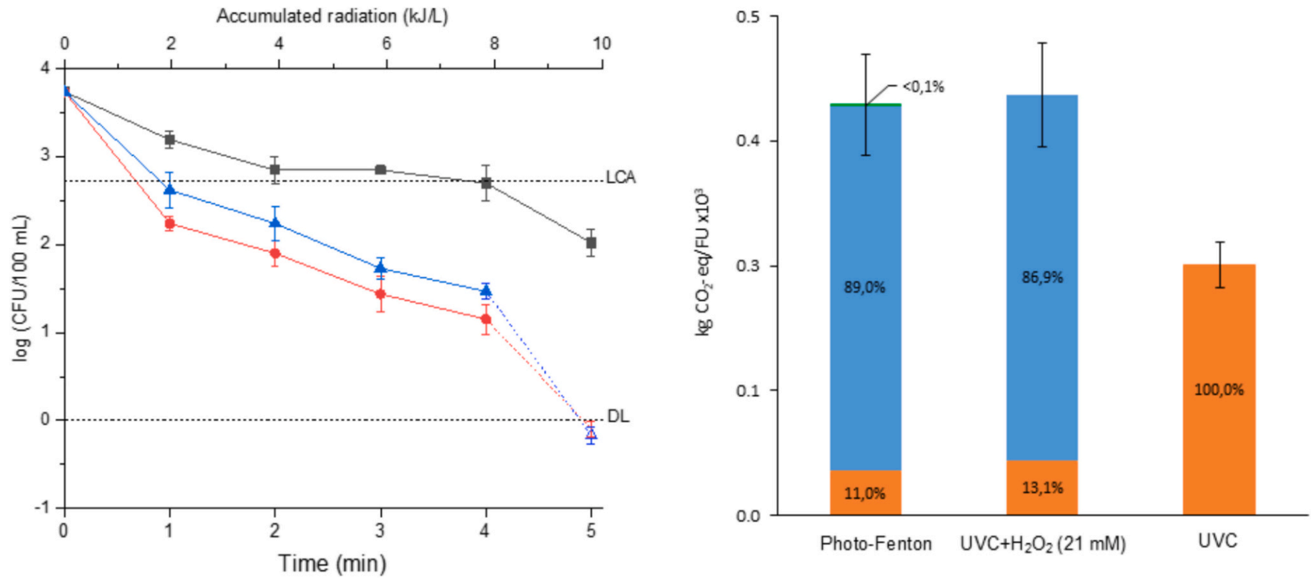

Fig. 5. a) *Clostridium* spp. inactivation by using WWTP1 (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) when treated with UVC (■), UVC + H_2O_2 (21 mM) (▲) and photo-Fenton process (UVC + H_2O_2 (21 mM) + Fe (11 μM) (●) and b) comparison of the environmental performances effects (GWP) of the Fe(II) (■), H_2O_2 (■) and energy (■). Method: ReCiPe 2016 (v1.08).

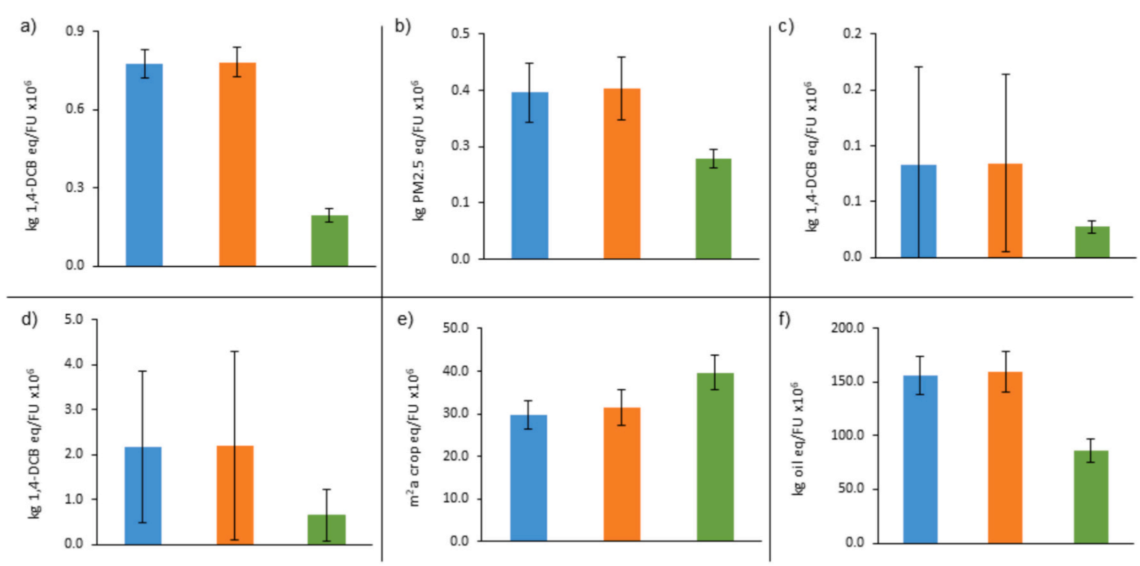

Fig. 6. Environmental impacts of *Clostridium* spp. inactivation when using WWTP1 (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) by photo-Fenton (■), UVC + 21 mM H_2O_2 (■) and UVC (■). From left to right: a) METP, b) PMFP, c) HTPc, d) HTPnc, e) LOP and f) FFP. Method: ReCiPe 2016 (v1.08).

(Fig. 7a), if the irradiation time is set at t = 5 min (same electricity consumption), the process is ruled by the amount of the added H_2O_2. Accordingly, the lower is the amount of H_2O_2, the lower is the GWP. However, also *Clostridium* spp. concentration only reached DL in some cases and data was calculated to decrease *Clostridium* spp. concentration by 1 LRV; also in this case, values were ruled by H_2O_2 addition and UVC seems to be the most sustainable.

The same impact categories analysed in the previous section are depicted in Fig. 8, while the whole set of results is reported in Table S3. Patterns similar to the ones observed in the previous section are observed since H_2O_2 resulted again as the main contributor. Uncertainty values are reported in Table S4.

3.3. *Application of LCA to CECs removal*

3.3.1. *Effect of the type of treatment*

However, to compare better the sustainability of the different treatments as quaternary processes, it has to be taken into account that disinfection is commonly a goal that is more easily reached than the removal of CECs. Therefore, reaching this goal might involve major environmental burdens. For these calculations, data related to the removal of acetaminophen with the same treatments and identical experimental set-up and published elsewhere have been employed (López-Timoner et al., 2023). Acetaminophen was chosen because it is typically found in WWTP effluents and shows a medium-high reactivity towards AOPs (Vallés et al., 2023). The initial concentration was 1000 μg/L (6.6 mM), as the total concentration of emerging pollutants has been reported to be several hundred μg/L (Yang et al., 2024). For these calculations, the reaction time to completely remove the

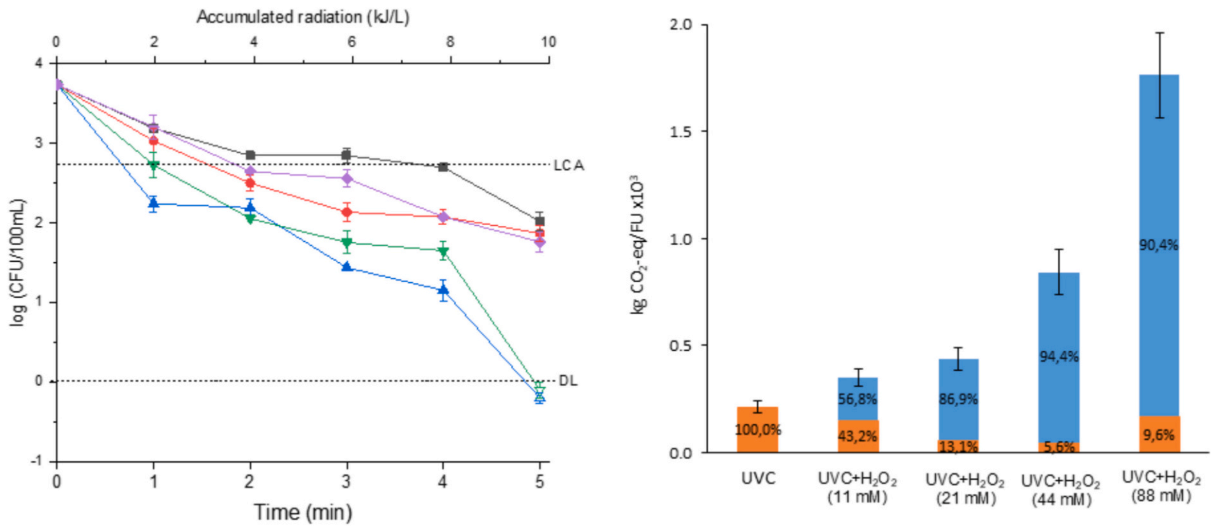

Fig. 7. a) *Clostridium* spp. inactivation when using WWTP1 (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) by UVC irradiation (■), UVC + 11 mM of H_2O_2 (●), UVC + 21 mM of H_2O_2 (▲), UVC + 44 mM of H_2O_2 (▼) and UVC + 88 mM of H_2O_2 (♦) and b) comparison of the environmental performances effects (GWP) by H_2O_2 (■) and energy (■). Method: ReCiPe 2016 (v1.08).

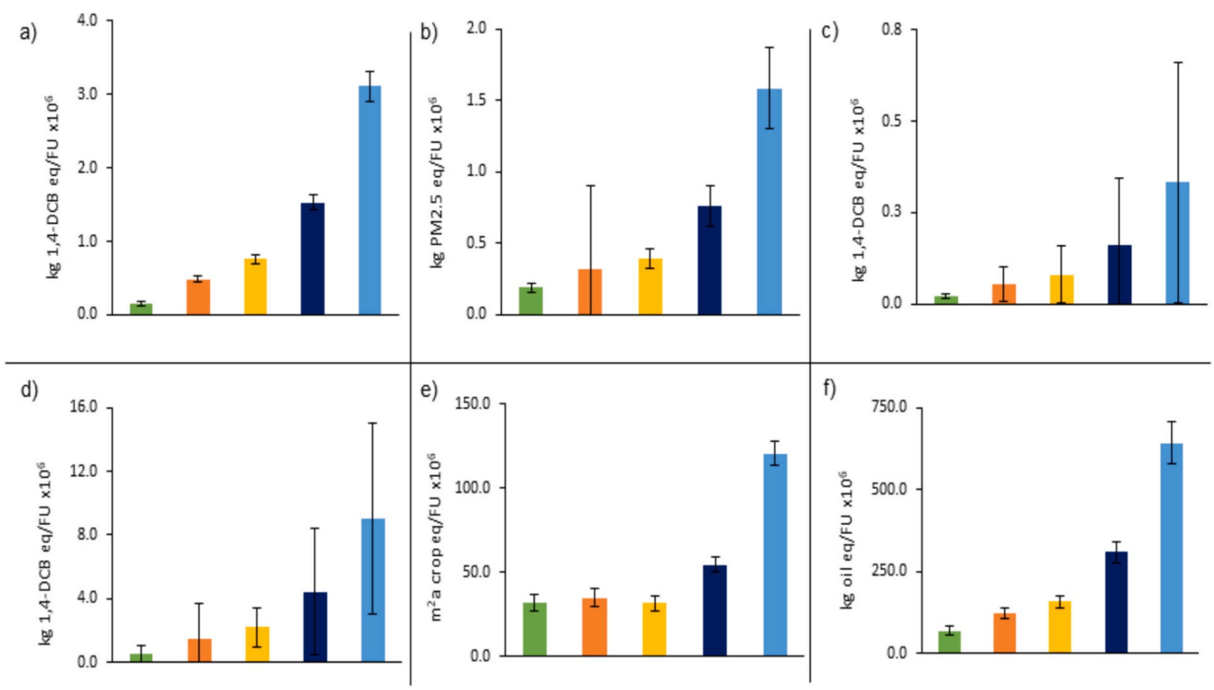

Fig. 8. Environmental impacts of *Clostridium* spp. inactivation when using WWTP1 (CFU_0/100 mL = 6.10 × 10^3; pH_0 = 7.5) by UVC (■), UVC +11 mM H_2O_2 (■), UVC + 21 mM H_2O_2 (■), UVC + 44 mM H_2O_2 (■) and UVC + 88 mM H_2O_2 (■). From left to right: a) METP, b) PMFP, c) HTPc, d) HTPnc, e) LOP and f) FFP. Method: ReCiPe 2016 (v1.08).

acetaminophen, as well as the added reagents were taken from previous work (López-Timoner et al., 2023), and they are shown in Table 3.

Fig. 9 represents the GWP estimated for the selected treatments. According to expectations, GWP for chemical treatments are significantly above those for *Clostridium* spp. disinfection (ca. one order of magnitude, 10^{-3} kg CO_2-eq/FU vs. 10^{-4} kg CO_2-eq/FU respectively). In this case, because of the higher amount of time required for the irradiation, electricity resulted to be the main hotspot. In line with this, the sustainability of the process is ruled by the irradiation time, despite some reagents being required, in sharp contrast with disinfection. In the case of acetaminophen, the lowest GWP is reached by photo-Fenton, which was the fastest process, followed by UV/H_2O_2 and then UVC. Regarding reagents, it is highlighted the high contribution for H_2O_2 when compared with iron (II), which was less than 1 %.

Also, in the others environmental categories investigated, photo-Fenton resulted in better environmental performances and electricity consumption resulted again the main contributors to the impact in all cases (see Fig. 10). More details are reported in Supplementary Materials (Table S5), where a second visualization is proposed for the same categories than represented before. Uncertainty values are reported in Table S6.

3.3.2. Hydrogen peroxide influence

GWP was also calculated for the UVC/peroxide processes at different

Table 3
Irradiation time required for the removal of acetaminophen under different conditions (López-Timoner et al., 2023).

	Treatment type	Complete removal time (min)
Effect of reagents	Photo-Fenton	15
	UVC + H_2O_2 (21 mM)	30
	UVC + Fe(II) (0.02 mM)	60
	UVC	60
Effect of H_2O_2 concentration	UVC + H_2O_2 *(11 mM)*	25
	UVC + H_2O_2 *(21 mM)*	15
	UVC + H_2O_2 *(44 mM)*	15
	UVC + H_2O_2 *(108 mM)*	15

concentrations of H_2O_2. It can be observed in Fig. 11 that there is an optimum point when 21 mM of H_2O_2 is used. It is worth noting that the optimum coincides with the shortest irradiation time as indicated in the previous paper (López-Timoner et al., 2023).

As in the previous case, the other impact factor categories are presented below. The complete set of results is reported in Table S7, while uncertainty values are in Table S8. Fig. 12 shows that studied environmental impacts increase with the addition of higher amounts of hydrogen peroxide. Thus, the optimum point from an environmental perspective is equal to that obtained with the degradation (in agreement with the previous results).

3.4. LCA of the experiments reported in this work

In the LCA calculations above reported, only the reactor was included in the system (scenario 2, see section 2.3.1), as the performance of the studied technologies was considered the most relevant aspect to evaluate the sustainability of the process. However, it also seems interesting to report on the sustainability of the experiments performed in this work and to identify those processes that have the highest impact (scenario 1). GWP for the disinfection and acetaminophen removal under UVC, UV/H_2O_2 and UVC-driven photo-Fenton can be found in Fig. 13. It is interesting that in both cases, differences among the treatments are negligible because, as indicated below, the major effect is not attributable to the treatment itself, but to sample transportation and the analytic stage.

In the case of *Clostridium* spp. disinfection, this process resulted in a GWP of 0.44 kg CO_2-eq/FU. It has to be indicated that 74 % of such value is due to energy consumed by the oven and the autoclave in the analytical step, thus explaining why energy is so predominant. By excluding this stage, 99 % of the total carbon emissions are derived from the transportation of the water matrix to treat from the WWTP to the laboratory. It should be noted that this value could be reduced by optimizing the process by which the water is transported. For instance, transporting larger quantities of water on the same route. Thus, reactions represent a very minor amount of the GWP: 0.1–0.4 × 10^{-3} kg CO_2-eq/FU vs. the global 0.44 kg CO_2-eq/FU, thus accounting for less than 0.1 % of the value.

In the case of acetaminophen in the same scenario, the calculated GWP was higher, 0.85 kg CO_2-eq. In sharp contrast with disinfection, reagent is the major factor in this case. This can be explained by taking into account that 70 % of the total GWP estimated is due to the raw materials used in the analytical step, mostly attributed to the acetonitrile used in the HPLC. By excluding this stage, again 99 % of the total carbon emissions derives from the transportation of the water matrix to treat from the WWTP to the laboratory, and less that 1 % is attributable to the reaction (1–3.5 × 10^{-3} kg CO_2-eq/FU vs. 0.85 kg CO_2-eq/FU).

4. Conclusions

UVC/H_2O_2 has been demonstrated to be the most efficient treatment to remove *Clostridium* spp. in WWTP effluents. However, application of LCA showed that UVC photolysis was more sustainable despite its lower efficiency. As treatment times were relatively short, highest impacts were due to addition of reagents. On the other hand, longer irradiation intensities required for chemical decontamination resulted in higher GWP values, which were ruled by energy consumption. Thus, circumneutral photo-Fenton was the most efficient for acetaminophen removal and showed lower environmental impact.

In order to make more accurate comparisons, the time and quantity of reagents required to decrease the concentration of *Clostridium* spp. by one order of magnitude was chosen as FU, as complete disinfection was not reached in all cases in the treatment time. Following the same procedure, the decrease of acetaminophen concentration from 1 mg L^{-1} to the detection was limit was chosen in the case of chemical

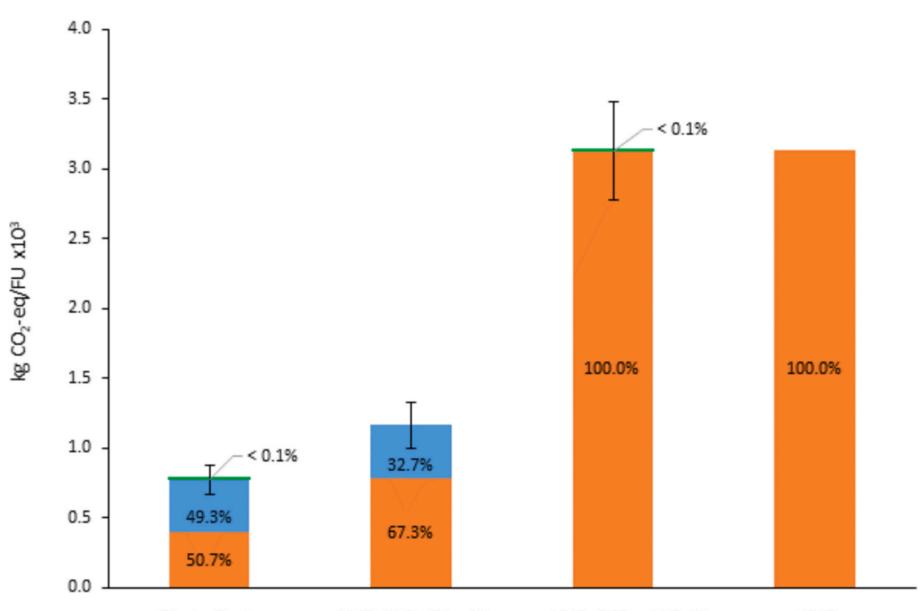

Fig. 9. Comparison of the environmental performances effects (GWP) for acetaminophen removal when using WWTP1 (C_0 = 1000 μg/L; pH = 7.5) by Fe(II) (■), H_2O_2 (■) and energy (■) using different treatment types (photo-Fenton, UVC + 21 mM H_2O_2, UVC + 0.02 mM Fe(II) and UVC). Method: ReCiPe 2016 (v1.08).

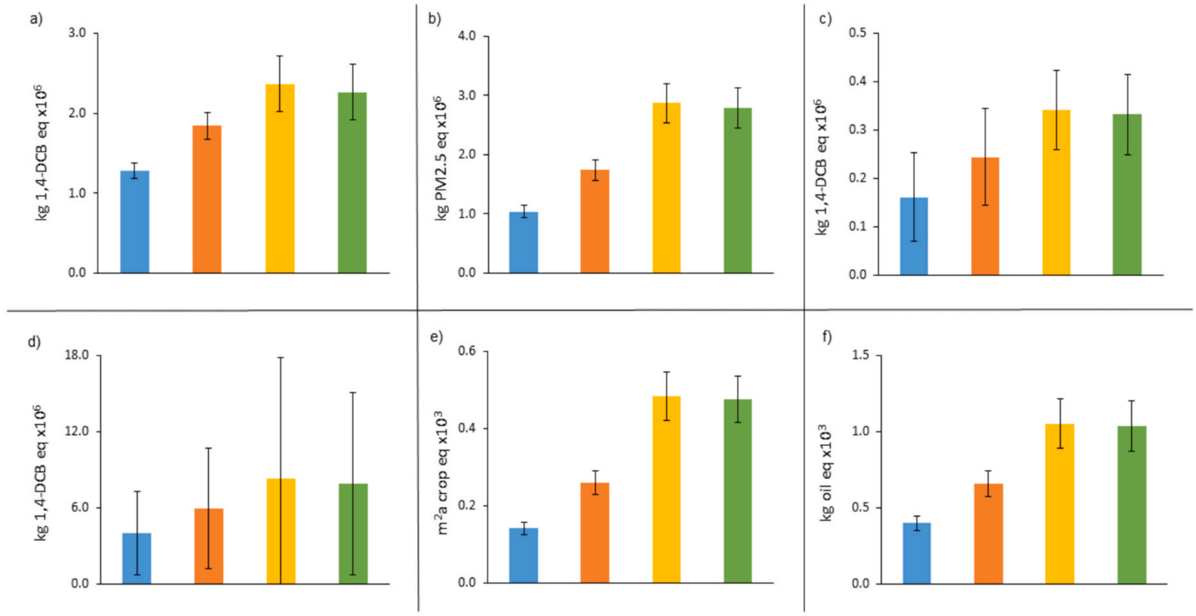

Fig. 10. Environmental impacts of the acetaminophen degradation when using WWTP1 (C_0 = 1000 μg/L; pH = 7.5) by photo-Fenton (■), UVC + 21 mM H_2O_2 (■), UVC + 0.02 mM Fe(II) (■) and UVC (■). From left to right: a) METP, b) PMFP, c) HTPc, d) HTPnc, e) LOP and f) FFP. Method: ReCiPe 2016 (v1.08).

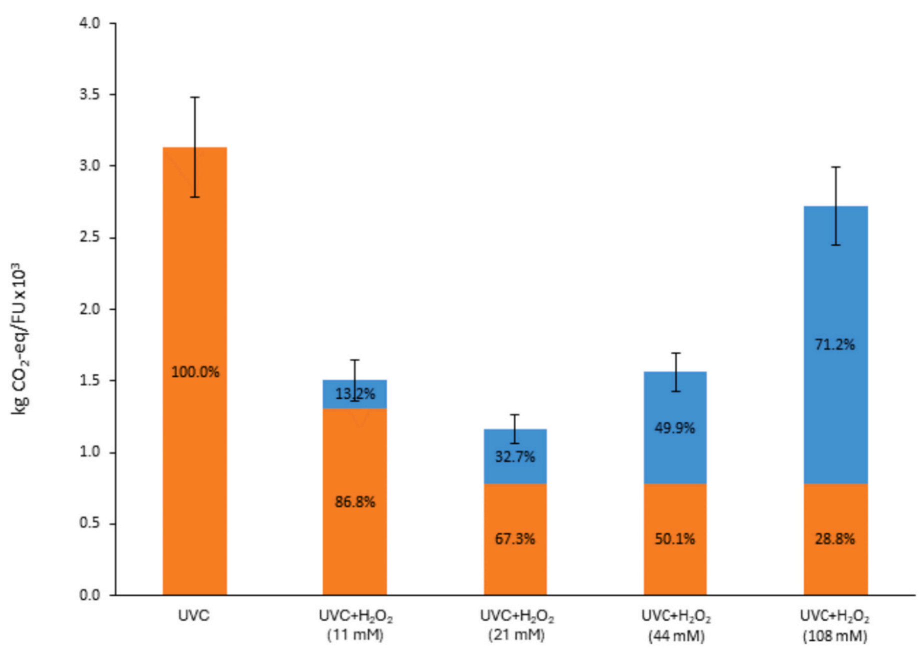

Fig. 11. Comparison of the environmental performances effects (GWP) for acetaminophen removal when using WWTP1 (C_0 = 1000 μg/L; pH = 7.5) by H_2O_2 (■) and energy (■) using different treatment types (UVC, UVC + 11 mM H_2O_2, UVC + 21 mM H_2O_2, UVC + 44 mM H_2O_2 and UVC + 108 mM H_2O_2,). Method: ReCiPe 2016 (v1.08).

decontamination. When both types of contamination (chemical and microbiological) are present, the major burden is attributable to chemical decontamination (ca. one order of magnitude above) and thus, this could be considered as the limiting step to determine the performance of a quaternary treatment.

Finally, LCA methodology was applied to the experimental work performed in the laboratory. Interestingly, the impact of the reactions themselves is negligible and the majority of the burdens are attributable to samples transportation and analysis. Thus, it is important to optimize these two operations, by minimizing the number of analyses and to place the additional plant near an already existing WWTP. In addition, as mentioned in Section 2.3.1, it must be recalled that the estimated impact of laboratory-scale systems is generally higher than that of industrial-scale systems. This consideration supports the potential implementation of the proposed system within an existing WWTP.

CRediT authorship contribution statement

R. López-Timoner: Writing – original draft, Investigation. **L. Santos-Juanes:** Methodology, Formal analysis, Conceptualization. **A.M. Amat:** Funding acquisition, Conceptualization. **F. Arfelli:** Methodology, Investigation, Data curation. **D. Cespi:** Supervision, Methodology, Data curation. **F. Passarini:** Supervision, Funding acquisition. **M.I. Polo:** Formal analysis, Conceptualization. **E. Zuriaga:** Methodology, Funding

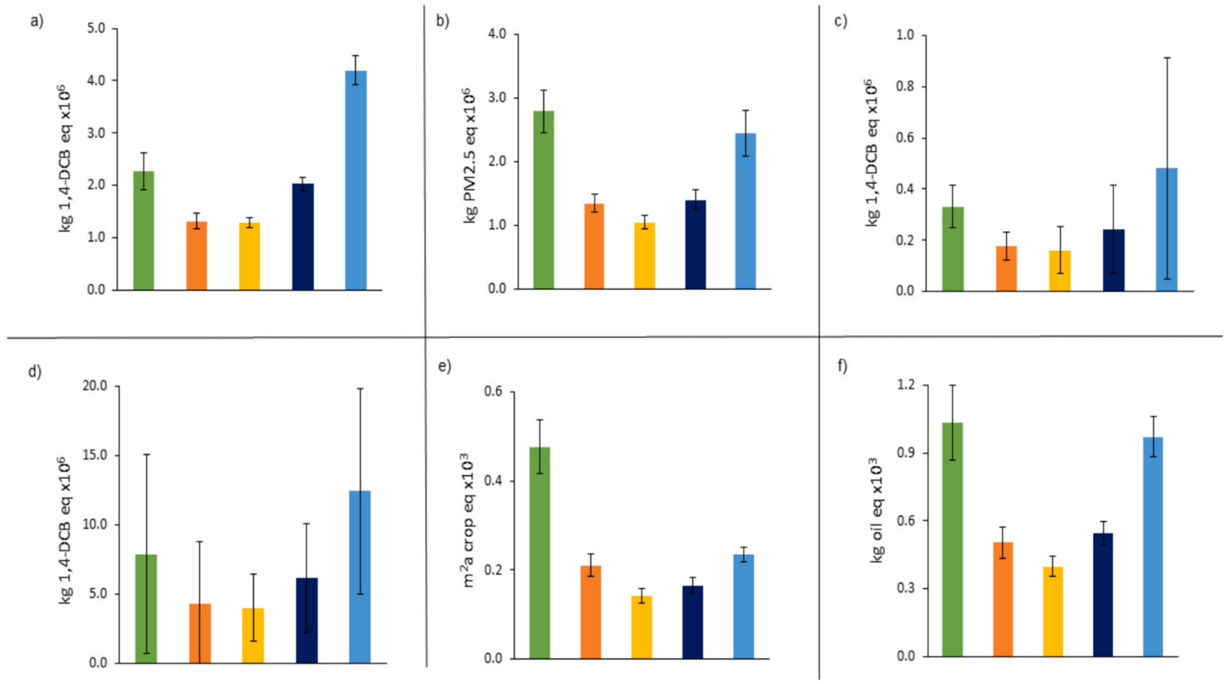

Fig. 12. Environmental impacts of the acetaminophen degradation when using WWTP1 (C_0 = 1000 μg/L; pH = 7.5) by UVC (■), UVC +11 mM H_2O_2 (■), UVC + 21 mM H_2O_2 (■), UVC + 44 mM H_2O_2 (■) and UVC + 108 mM H_2O_2 (■). From left to right: a) METP, b) PMFP, c) HTPc, d) HTPnc, e) LOP and f) FFP. Method: ReCiPe 2016 (v1.08).

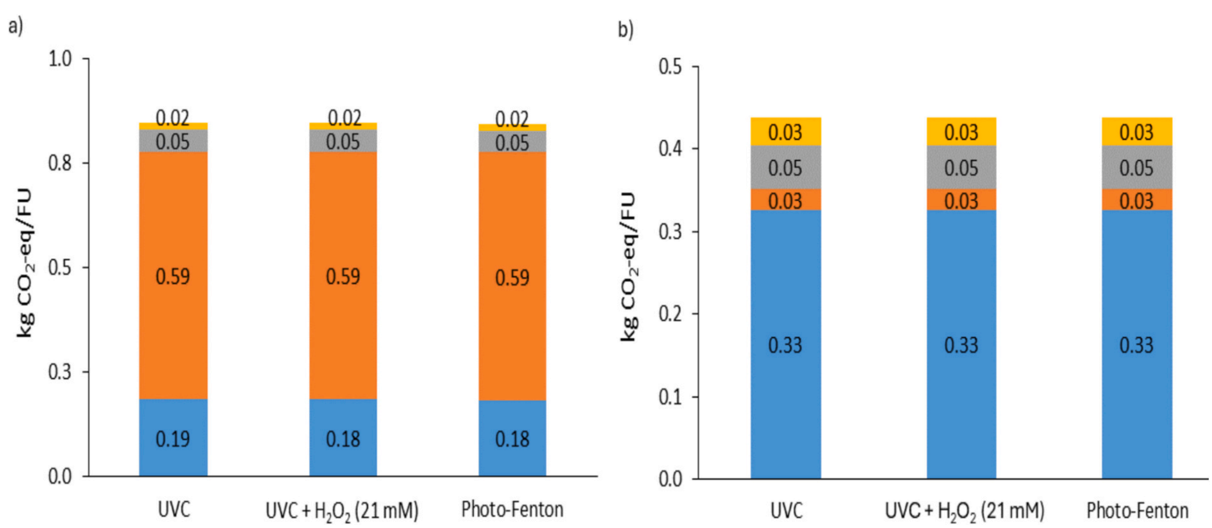

Fig. 13. Total GWP comparison related to energy (■), raw materials (■), transportation (■) and waste (■) for a) acetaminophen removal (C_0 = 1000 μg/L) and b) *Clostridium* spp. (CFU_0/100 mL = 6.10 × 10^3) disinfection under same operation conditions when using WWTP1 (pH = 7.5).

acquisition. **A. Arques:** Writing – review & editing, Supervision, Funding acquisition.

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.

Acknowledgements

Funded by European Union NextGenerationEU with the support of Ministerio de Ciencia e Innovación – Spanish Government (TED2021-130994B-C32 (Ecotranseas)).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.179029.

Data availability

Data will be made available on request.

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