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“Integrated water cycle sustainability: sonozone technology for the water reuse in a circular economy perspective”

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LIST OF ACRONYMS AND ABBREVIATIONS:

AC: activated carbon

ACS: activated sludge process

AOP: advanced oxidation process

BOD: biochemical oxygen demand

CEC: contaminants of emerging concern

COD: chemical oxygen demand

DBP: disinfection by-product

DO: dissolved oxygen

DW: drinkable water

DWW: drinkable wastewater

E. Coli: Escherichia Coli

EC50: effective concentration

GC-MS: gas chromatography – mass spectrometry

GHG: greenhouse gasses

HL: half-life

HM: heavy metal

HPLC-HRMS: high-performance liquid chromatography - high resolution mass spectrometry

H-T: high toxicity

IC: inorganic carbon

ICP-AES: inductively coupled plasma - atomic emission spectrometry

IWC: integrated water cycle

IWW: industrial wastewater

LC50: lethal Concentration

L-T: low toxicity

M-T: medium toxicity

N.T.: non treated

NDIR: nondispersive infrared analyzer

NOEC: no observed effect concentration

nP: non-persistent

NPOC: non-purgeable organic carbon

nT: non-toxic

O_3: ozone

P.E.: population equivalent

P: persistent

PEff: primary effluent

PFAS: per-fluoro alkyl substances

PP: pilot plant

RWW: reclaimed wastewater

S. Enteritidis: Salmonella Enteritidis

spp.: several species

SW: storm water

TC: total carbon

TDS: total dissolved solids

TN: total nitrogen

TOC: total organic carbon

TP: total phosphate

TSP: triple super phosphate

TSS: total suspend solids

UN: united nations

US: ultrasound

UV-Vis: ultraviolet and visible

UWW: urban wastewater

vP: very persistent

VSS: volatile suspended solids

WW: wastewater

WWTP: wastewater treatment plant

1 ABSTRACT

The work that is presented aims to be a preliminary study on the applicability and potentialities of a low-frequency ultrasonic treatment combined with an ozone disinfection on a primary effluent from an urban wastewater treatment plant for an agricultural reuse (fertigation). The preservation of the nutrients contained in the original wastewater may contribute to the circular economy perspective, reducing the production costs for mineral fertilizers and the freshwater withdrawals. The limited scientific experience towards the hybrid ultrasonic and ozone process, called sonozone, requires an accurate laboratory experimental campaign, focused on the assessment of the regulated physico-chemical and biological compounds, that have to be controlled and maintained below the legislative limits. Particular attention has to be put on the pathogenic inactivation, as the reclaimed wastewater must be safely applied on crops. The technical feasibility will be associated with an economic assessment and an ecotoxicological evaluation. The importance of the wastewater recovery is nowadays increasing due to the climate change and the population growth. The integrated water cycle sustainability should move closer to the "zero-waste" concept and expand the classic wastewater treatment plant depuration towards resource recovery. The suitable application of the reclaimed wastewater for fertigation practices could help the process to move towards the integrated water cycle closing, following the circular economy perspective.

The primary effluent was appositively selected for the reuse. The solid load of a wastewater treatment plant inflow, from the drainage system, may be unsuitable for the direct sonozone treatment; the larger solids will promptly cause the clogging of the pipes and large diameters of particles may be unsuitable for the designed treatment times. So, the selected primary effluent is the wastewater deprived from these solids but unaltered in the dissolved properties. The maintenance of the dissolved nutrients is paramount for the current study purpose.

The work started with a laboratory-scale calibration of the instruments (ultrasonic probe and ozone generator) separately. An initial semi-continuous ultrasonic process and a discontinuous ozonation were performed. A precise characterization of the primary effluent wastewater was needed before the experiments. Then, the combination of processes in a semi-discontinuous setup allowed to test the coupled effect of the sequential ultrasonic pretreatment and ozonation. The hybrid process' high removal efficiency was matched by an outstanding retention of nutrients (total nitrogen and total phosphate) highlighting the potential for the primary effluent reuse, with possible significant saving of chemical fertilizers.

ABSTRACT

The wastewater' sonozone recovery could save a meaningful amount of nutrients. However, being a well-known energy-consumer processes, it has to be assessed on the field based on the removal capacity. An economic assessment was given for the hybrid technology and compared to other classic secondary and tertiary treatments. Towards a safe microbial recovery, an in-depth analysis on microorganisms was presented. Being only few the mandatory pathogens required in the legislation, additional microorganisms will be tested with the ultrasonic and ozone processes alone and combined. The kinetic modelling and logarithmic abatement of four microbial species, namely *Pseudomonas* spp., *E. coli*, *Enterococcus* spp. and *S. Enteritidis*, was performed. Moreover, an ecotoxicological in-silico evaluation indicated the sonozone removal capacity towards several hazardous and persistent chemicals, addressing the eco-toxic concentration in different environments, their persistence and solubility in water.

The pilot scale laboratory tests should be intended as the transition from a semi-continuous laboratory scale to a bigger-scale plant running continuously, as a further step for the evaluation of the proposed agricultural reuse. The reactor building and the connection of the units will be described, additional flow dynamics are design and tested. The wastewater treatment through the pilot unit will be matter of future analysis.

The circular economy concept is strongly related to the possibilities of the conventionally removed compound to be recovered and reused through the fertigation concept. In order to support the laboratory tests, a meaningful case study was analyzed. Two different scenarios were shown for the extent of expressing a tangible recover of water and nutrients. However, stakeholders and citizens are still reluctant in the adoption of this wastewater reuse practice for the irrigation, and thus proper informative campaign to relevant stakeholders should be always planned before moving to full-scale considerations.

The overall study outcomes showed remarkable opportunities for the primary effluent reuse for agricultural purposes in a circular economy perspective, despite energy costs may still hinder the full-scale applicability of this technology. Moreover, the sonozone application in rural areas, not reached by a suitable sewage and depuration system, may result highly recommended for the prospective of direct effluent reuse, especially when coupled with an electricity generator plant (e.g. photovoltaic panels).

2 INTRODUCTION

The extensive use of high-quality water, in addition to the non-stopping population increase and the inexorable climate change, may lead to severe reduction in global water availability [1]. The urban, industrial, and agricultural sectors are the main freshwater consumers, reaching about four trillion cubic meters annually [2]. Water usage was increasing, during the last century, at a rate twice as the population growth [3]. Forecasts are projecting that the water demand may be 55% higher in 2050 than in 2015, due to the increased utilization in manufacturing, electricity generation, and household applications [4]. United Nations (UN) drafted in 2015 the “2030 Agenda for Sustainable Development”, adopted by all the UN members [5]. Considering water exploitation consistent with the predictions, worldwide the countries are not prepared to achieve the United Nations Sustainable Development Goal 6 (Fig. 2-a), which aspires to accomplish extensive and sustainable water management and sanitation by 2030 [6]. To satisfy this intent, the entire world must move from the current situation to a new one, with several additional investments in the sectors related to wastewater (WW) treatment and recovery. Above all, the most pressing sector towards the freshwater supplies is the agricultural one. We may assume that the 70% of worldwide freshwater withdrawal is used in the agricultural sector [7]. Irrigation with reclaimed wastewater could be an alternative method to reduce the freshwater storage's pressure. Moreover, there is a necessity to safely introduce a circular reuse of reclaimed water in other daily practices where freshwater is commonly used. The most important necessity is to guarantee the desired quality of recycled water to be harmless for every human being and for the environment [8]. Reusing the reclaimed wastewater for agricultural purposes could be a fair practice in a context of freshwater availability’s lacking and climate change, which leads to more severe drought seasons [9].

Figure 2-a: SDGs

2.1 Integrated Water Cycle explanation and technologies

The concepts that are going to be explained in this chapter are mandatory towards an understanding of a sustainable and environmental-friendly wastewater recovery. Different sources are causing a pollution inside the water, requiring a proper assessment and management of the waste products. The wastewater characterization and treatment have to be considered and designed. Nowadays, diverse technological processes are available but not all of them are suitable for a recovery of the wastewater. The study will mostly focus on the assessment and treatment of the urban wastewater. Anyway, it can be applied also to the industrial wastewater flows. The legislative thresholds that have to be respected will be reported as well.

2.1.1 Integrated Water Cycle definition

The Integrated Water Cycle (IWC) refers to the sequence of three main water services: Aqueducts, Sewage systems and Depuration (Fig. 2-b). The aqueduct is the initial part of the IWC and consists of processes such as the catchment, the first potabilization treatment and the distribution of the water from the source to the consumers. The withdrawal from the environment may be different, depending on the source from which the water is withdrawn. Springs, artesian wells, rivers, lakes, reservoirs and surfaces aquifers are the most accessible catchment points. Successively, the collected water has to be stored in proper reservoirs and purified, in order to meet the minimum quality required by the national or international drinking guidelines regarding physical, chemical and biological parameters. Through a designed system of pipes, the drinkable water (DW) is transported in each area belonging to the network district, adopting pressurized flows. During the distribution of the purified water to the final utilities, it is mandatory to preserve the characteristics of the water.

Then, the used water employed for any purpose by the household or industrial users have to be gathered and transported to the wastewater treatment plants (WWTPs) through the drainage system. The water, from here on, has to be called WW due to the changing in its properties after the contact with some anthropomorphic and mechanic utilizations. This WW must be properly treated in the WWTPs and discharged in specific areas (surface basins, soils or collected in further drainage systems) in order to return it quantitatively and "approximately" qualitatively to the environment [10]. The qualitative assessment will be better explained in the following chapters, anyway the possibility to "close" the IWC is technically feasible, however the economic convenience is still not satisfactory in order to generally apply the process in full scale operations.

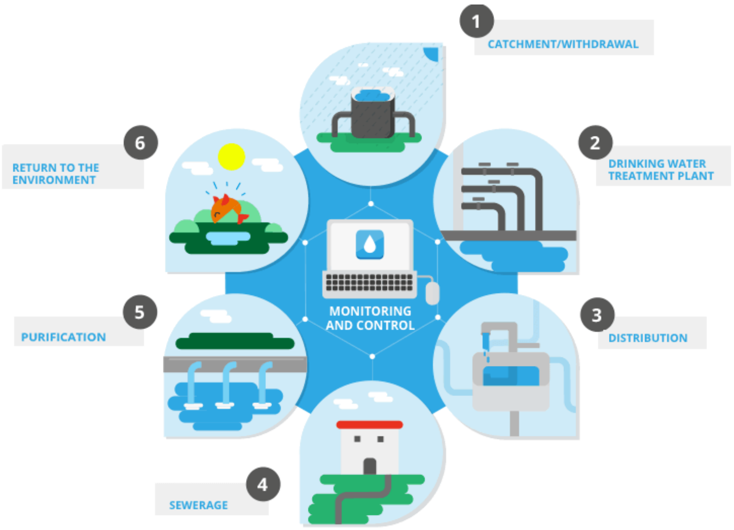

Figure 2-b: IWC structure

In order to avoid issues in the planning of the processes, often the entire IWC is ruled by the same managing authority [11].

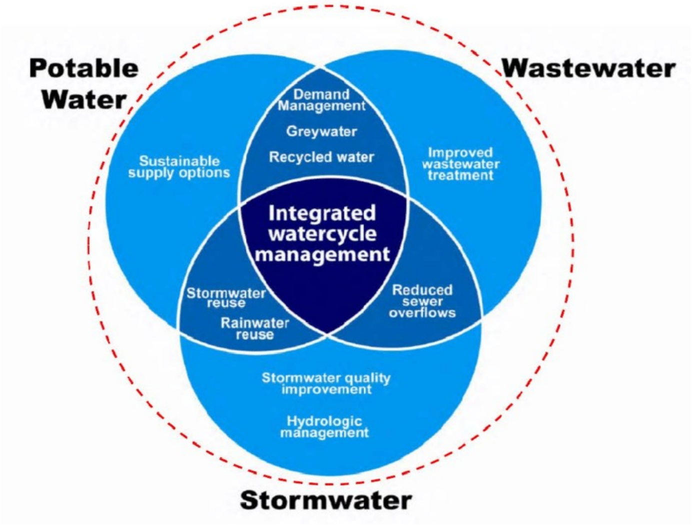

Figure 2-c: IWC conceptual scheme

The improvement of a sustainable and efficient DW and WW management is one of the most important aspects requested by the SDGs. As shown in the Figure 2-c, in the same way as DW and WW, the stormwater (SW) has to be considered. In particular, from an optimal IWC management point of view, the initial part (the first 2 mm of rainfall) of the SW that reached the soils has to be considered as strongly polluted water that must be treated in the WWTPs [12].

2.1.2 Wastewater Characterization

The following two types of WW are distinct from one another: domestic wastewater (DWW) and industrial wastewater (IWW). DWW is defined as water altered in quality after being in contact with anthropic activities, such as human metabolism and household uses, whereas IWW is discharged from other possible processes, mainly industrial ones. The mixture of these two different wastewaters and the run-off stormwater is called urban wastewater (UWW).

The composition and the load of the organic matter, solids, nutrients, micropollutants (such as heavy metals, pesticides, drugs, surfactants, pharmaceuticals and more) and microorganisms may vary based on the different WW sources. Large differences in the properties (amounts and types of contaminants) of DWW, IWW and UWW result in challenges with the measurements [13]. These heterogeneities may require different designs of the measurement campaigns and equipment used for characterization. The prevailing pollutants included in the WW are summarized in Table 2-1.

Table 2-1: Main WW pollutants and typical concentration in Urban WWTPs

Category	Parameter	Acronym	Typical concentration in urban WWTPS	Reference
Physicochemical	Alkalinity	-	100-200 mg $CaCO_3$/L	[14], [15]
	Conductivity	-	600-800 mS/cm	[16]
	Dissolved Oxygen	DO	5 mg/L	[17]
	pH	-	7-8	[18]
	Total Hardness	-	300 mg/L	[15]
	Turbidity	-	50 NTU *	[19]
Organic matter	Biochemical Oxygen Demand	BOD	80-300 (BOD_5) mg/L	[18], [19]
	Chemical Oxygen Demand	COD	160-500 (Total) mg/L 200 (Soluble) mg/L 300 (Suspended) mg/L	[16]
	Total Organic Carbon	TOC	30-200 mg/L	[17]
Solids	Total Dissolved	TDS	600 mg/L	[17]
	Total Suspended	TSS	200-400 mg/L	[16]
	Volatile Suspended	VSS	320 mg/L	[16]
Nutrients	Nitrogen	N	60–110 (TKN) mg/L 50-100 (NH4-N) mg/L 0,5 (Nitrate + Nitrite) mg/L 25 (Organic N) mg/L	[16], [18]
	Phosphate	P	15 (TP) mg/L 10 (Ortho-P) mg/L 5 (Organic P) mg/L	[16]
	Potassium	K	9 mg/L	[19]
Heavy metals (HMs)	Aluminum	Al	0,6 mg/L	[16]
	Cadmium	Cd	0,002 mg/L	[16]
	Chromium	Cr	0,025 mg/L	[16]
	Copper	Cu	0,07 mg/L	[16]
	Lead	Pb	0,06 mg/L	[16]
	Mercury	Hg	0,002 mg/L	[16]
	Nickel	Ni	0,025 mg/L	[16]
	Silver	Ag	0,007 mg/L	[16]
	Zinc	Zn	0,2 mg/L	[16]
Microorganisms	Coliphages	-	10^5 CFU/100 mL **	[16]
	Escherichia Coli	E. Coli	10^7 CFU/100 mL **	[16]
	Roundworms	-	10 eggs/L	[16]
	Salmonella	-	150 CFU/100 mL **	[16]
Surfactants	Total	-	13 mg/L	[19]

* NTU: Nephelometric Turbidity Unit

** CFU: Colony forming unit

2.1.3 Wastewater Treatment Plant: structure and treatments

The typical urban wastewater treatment plant is built as Fig. 2-d. The WW inflow, coming from the sewage system, is treated through a combination of different processes (physical, chemical and biological) for pollutants removal [20], [21]. The size of the WWTP is defined as the number of Population Equivalent (P.E.) that have to be treated throughout the plant. The WWTP possess two main lines: the water line (light blue arrows Fig. 2-d) and the sludge one (brown arrows in Fig. 2-d). In the current work we will focus only on the WW treatment, not mentioning at all the sludge line.

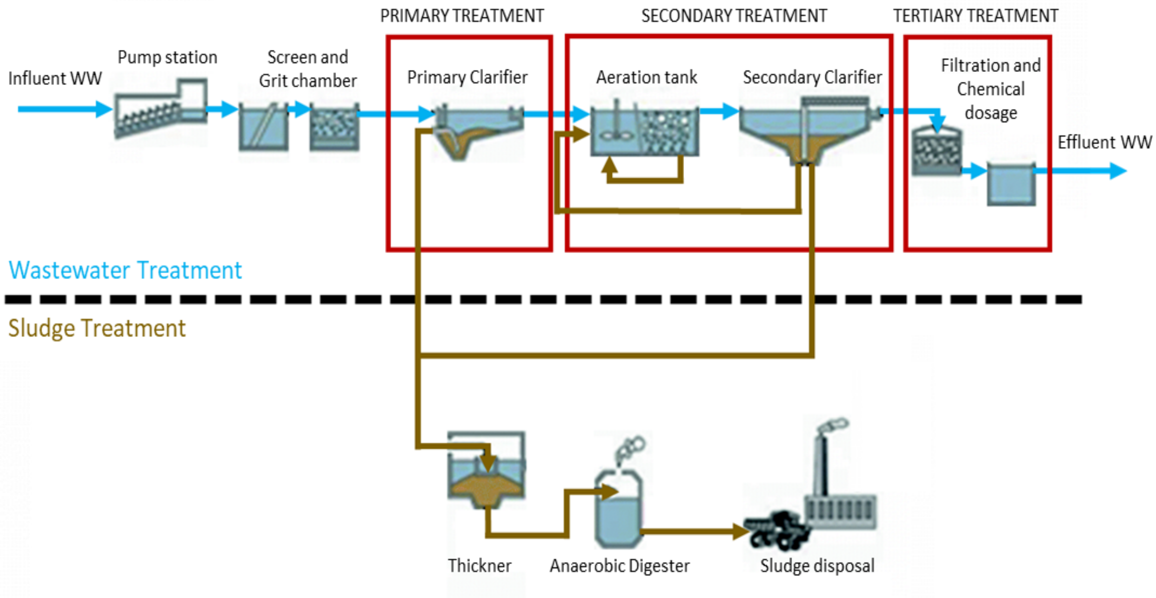

Figure 2-d: Typical WWTP layout

The inflow WW often requires a pumping station due to the not favorable slope of the ground or different heights from the soil level and the tank entrance. The initial pretreatment of the WW inflow is composed by a mechanical screening and a grit chamber, for the removal of the biggest solids and the technical sands (> 2 mm of diameter). In addition to them, oil and grease are occasionally removed in the same basin. The totality of these wastes is collected and transported into proper disposal areas. Successively, the WW arrives to the first settler tank, the primary clarifier. The remaining settleable solids are here removed from the bottom of the reactor and pumped to the sludge line. The clarified water, from the upper layers, is further pumped in the secondary treatment, the so-called Activated Sludge (ACS) Process [22]. A multi-chamber aeration tank is connected to a secondary clarifier, with the addition of an internal (aerobic to aerobic) and an external (clarifier to aerobic) recycle underflow. The ACS is the most diffused depuration technique towards the nutrient and organic matter remotion, caused by the microorganism's action. The design of the big size WWTPs, having a potentiality larger than 100.000 P.E., always requires a series of aerobic and anaerobic/anoxic basins. The sequence of an aerobic and an anoxic reactor is widely adopted for the nitrogen removal (Nitrification and Denitrification) and the anaerobic sector may stress the microorganisms and allow the phosphate removal (Dephosphorylation) to be more efficient. Anyway, all these additional biological treatments are included in the ACS process concept. The active biomass coming from the aerobic section is gathered, after its settling, from the bottom of the secondary clarifier and recycled. An optional tertiary treatment, used when needed, is composed by chlorination, UV irradiation, Advanced Oxidation Processes (AOPs), filtration processes, such as Activated Carbon (AC), Sand filters, Reverse Osmosis and other chemical dosages [23]. The aim of the tertiary treatment is the remotion of residual pathogen organisms, hazardous for the human health and the environment balance. The conclusion of the depuration line is the discharge of the treated WW to the desired location.

The ACS treatment, as abovementioned, is a robust and widely employed methodology for the removal of different pollutants. Moreover, it is adopted from more than 100 years in the WW treatment sector. However, the world nowadays requires to push towards a nutrient recovery and reuse, not only to the respect of strict depuration thresholds.

2.2 A concrete application for a suitable WW recovery

The fundamental nutrients contained in the wastewater flows, basics for the vegetation growth, are nitrogen, phosphorus and potassium (Table 2-1). A fertigation practice is defined as the application on soils of nutrients that were previously mixed and dissolved in water. However, this methodology is frequently interpreted as fresh water combined with granular fertilizers and spread on the soils through a piping system. Fertilizers are essential as a food supply in intensive agriculture; nonetheless, they contribute to GreenHouse Gasses (GHG) emissions and environmental dispersion, with adverse environmental effects [24]. In order to satisfy the current project’s WW recovery idea, a different approach have to be intended. If the N-P-K concentration in the WW is acceptable, a certain recovered WW (RWW) volume could be directly pumped onto the crops, establish the fertigation practice in the same way. This volume had to be previously treated in the upstream WWTPs, e.g., by adopting further designed disinfection processes. Fertigation could avoid excessive nutrient leaching since this methodology of nutrient distribution promotes a rapid crop’s uptake.

Nitrogen is the most abundant nutrient. Its use in industrial fertilizers is associated with high dispersion that can induce surface and ground water pollution, eutrophication, ammonia and nitrates contamination [25]. These losses limit its utilization by crops, could be finally harmful to humans and reduce overall crop productivity [26].

Phosphorous, a nutrient which global geological reserves are limited, and undergoing a fast depletion [27], could also be recovered from wastewater streams significantly enhancing the IWC sustainability [28], [29]. P recovery through struvite precipitation has been investigated as a recovery option, especially from anaerobic effluents and sewage sludge [30], [31]. However, a more direct solution would consist in direct effluent reuse for fertigation, exploiting effluents without (or with limited) P removal.

Potassium will be evaluated in the following section, as well as calcium (Ca) and magnesium (Mg). However, compared to N and P, their importance is slightly lower for the plant growth.

Specific effluent properties ought to be thoroughly assessed when implementing water reuse schemes to verify the fulfillment of fertilization requirements, adjusting the ratios if necessary [32]. Crops are characterized by different water volumes (m^3/ha yr) and nutrient mass (kg/ha yr) requirements. Punctual nutrient requirements should be considered in all fertigation approaches, comparing effluent characteristics with temporal crop needs [33]. Also, recommended N-P values vary depending on soil characteristics and baseline concentrations. Particular care, finally, should be posed in fertigation practices in nitrate vulnerable areas, such as areas at risk from agricultural nitrate pollution [34].

Fertigation is more attractive in situations of severe water scarcity, hence water-saving distribution systems (e.g., micro-sprinklers or subsurface drip irrigation) may be preferred to traditional ones (e.g., flood or sprinkler irrigation) in many situations.

Beside agronomic aspects, fertigation practices should include in-depth assessment of long-term effects on possible pedologic modifications. Depending on circumstances, RWW fertigation was shown to increase soil fertility, enhancing crop yield and quality, without alteration of aggregation, penetration resistance, water infiltration rates, porosity, and organic carbon content of the agricultural soil even after prolonged application [35].

Despite being present at very low concentrations in treated effluents (down to few ng/kg), Contaminants of Emerging Concern (CECs) can be harmful for plant growth as well as for humans and animals that consume them [36]. This aspect has to be considered during the RWW application on soils.

In conclusion, fertigation affects many different aspects of agricultural activities: these include agronomy, soil structure, environmental impacts, economic sustainability, social acceptance. The proper application of the fertigation practice could help the process move towards to the IWC closing and into the circular economy perspective.

2.3 Methods for the wastewater recovery

Above all the described WW treatments, it is possible to classify the totality of them into three main groups of processes: the physical, the chemical and the biological ones [37].

The typical physical process used in the WW sector include absorption, sedimentation and membrane filtration. All these processes are mostly effective for the removal of inorganic pollutants. Moreover, longer retention times need to be considered in order to let them be efficient. The pretreatment grit chamber, screenings and the clarifiers are examples of physical processes commonly adopted in the WWTPs.

A range of chemical processes are available, such as adsorption, chemical coagulation and flocculation. The specificity of them permits their employment for a wide assortment of targeted pollutants. Furthermore, a new class of chemical processes, the AOPs, has gained interest from the scientific and industrial communities due to their remarkable capacity of abatement of refractory organic pollutants. Further explanation will be given in the following chapters.

The biological processes rely on microorganisms for the elimination of organic compounds. They can be divided into two main groups: aerobic and anaerobic ones. The ACS process has been conventionally used for the organic abatement from wastewater streams. However, its applicability is limited for the remediation of organic CECs such as pharmaceuticals and personal care products [38].

The biological processes, mainly related to the ACS treatment in the WWTPs, are not suitable for the purposes of this study, due to the unconditional remotion of the nutrients from the WW. On the other hand, the physical process could not treat, by themselves, the WW enough in order to maintain the concentration of several compounds (such as pathogens) below the required thresholds of pollution, such as pathogens. So, the chemical treatments stand as particularly meaningful for the study.

2.3.1 Advanced Oxidation Processes (AOPs)

AOPs can remove a wide range of pollutants in wastewater and achieve high-level disinfection by promoting the generation of strong oxidants (mainly OH-radicals or other species) with nonselective oxidation and degradation of organic compounds and pathogens destruction [39]. The hydroxyl radicals (OH•), targeting the organic compounds, transform them into smaller and easily biodegradable organics or completely mineralize them into carbon dioxide and water. AOPs are indicated to remove refractory contaminants that cannot be destroyed using traditional technologies [40]. Despite complete pollutant mineralization may be economically unsustainable, combination of chemical and biological treatments can be feasible when dealing with concentrated agro-food industrial effluents [41], [42]. However, even AOPs proved to be ineffective for the degradation of highly refractory pollutants, such as Per-Fluoro Alkyl Substances (PFAS), that may be present at low concentrations in many types of WW [43]. Moreover, the high energy consumption and the installation costs are severe drawbacks. The AOPs applications for agricultural reclamation of wastewater are limited; the most relevant outcomes are summarized in Table 2-2.

Table 2-2: Advanced Oxidation Processes application for wastewater agricultural reuse

Single process	Treated stream	Main outcomes	Reference
Ozonation	Cattle wastewater	Excellent oil and greases (100%), Coliforms (99%), color (88,5%) and turbidity (93,4%) abatement after 2 h ozonation; lower COD (55%) and BOD (64%) removal	[44]
Ultrasounds	Urban wastewater	Degradation of organic compounds; mostly adopted as a pretreatment	[45]
Microwave assisted	Municipal Wastewater	Degradation of organic compounds; mostly adopted as a pretreatment	[46]
Ultraviolet (UV)-light emitting diode (LED) disinfection	Biological wastewater treatment plant (WWTP) effluent	Advantageous alternative to mercury-vapor lamps; UV-LED effluents do not affect soil organic matter composition for agricultural reuse	[47]
Photochemical electrolysis	Urban wastewater	Exploitation of renewable solar light; feasible disinfection option; more sustainable treatment chain	[48]
UV/H_2O_2, O_3/H_2O_2, electrochemical oxidation	Contaminated wastewater	UV/H_2O_2 and O_3/H_2O_2 achieved 100% compound degradation after 2 h; electrochemical oxidation showed slower degradation; eco-toxicity concerns	[49]
Fenton like	Leachates and strongly polluted wastewater	Organic compound removal after 30 min at 70 °C, heterogeneous phase reaction could enhance the efficiency	[50]

Among AOPs, ozone is particularly indicated for wastewater disinfection, being able to inactivate pathogens, due to its strong oxidative power [51]. Combination of different AOPs can lead to superior results when compared to individual processes [49], [52]. For the extent of this study, the attention will be given exclusively to the sonication and ozonation treatments.

2.3.1.1 Ultrasonic treatment

Ultrasound (US) generates the cavitation phenomenon, that is responsible of a mechanical, physical and chemical effects that may cause microorganisms' death inside a liquid medium [53]. The creation and powerful implosion of microbubbles result in a perturbation of the external environment [45]. During a sonochemical treatment, the bubbles' collapse generates high temperatures (> 5000 K) and pressures (> 1000 bar). Moreover, the highly oxidative radical specie of OH• is produced, which can target the organic pollutants and disrupt bacterial membranes, thus leading to microorganisms' death [54]. Despite the disinfection efficacy, full-scale applications of ultrasonic treatments require a huge amount of energy, resulting in high maintenance costs [55].

2.3.1.2 Ozone treatment

Ozone (O_3) is a strong oxidant, well-known in literature and widely applied in WW treatment [39]. It is recognized as a valuable disinfectant due to the capacity of generate free hydroxylic radicals. Ozone disinfection of WW from primary treatment could maintain the nutrients and, at the same time, be an efficient pathogen removal agent [56], [57]. The O_3 solubility in water is strongly related to the temperature and the pH [58]. Being twice as high at 20 °C than at 40 °C, a temperature control system must be installed while working with the ozone as an oxidant. Needing a shorter process duration, compared to the other above-mentioned AOPs, it can be helpful towards a reduction of the energy inputs. The Disinfection by-products (DBPs) are a serious matter of concern, particularly since disinfection is a key component of the necessary treatment train prior to RWW fertigation. Well-known ozone formed compounds, being in contact with the residual organic matter contained in the WW (humic and fulvic acids), are the aldehydes [52].

2.3.1.3 Hybrid AOPs: Combined US + O_3

From here on, the combination of US and O_3 will be called as Sonozone. This term does not have to be confused with the contemporary application of the two AOPs inside the same reactor; in this study the application of them will be exclusively indicated the sequence of US followed by the O_3.

The Sonozone potential benefit is related to the augment of the concentration of hydroxylic radicals, formed by the ultrasonic pretreatment, that enhance the efficiency of the process due to higher transfer rates into water [59]. Recent studies have proven an increase, during the combined process, of the organic matter removal [60]. Moreover, besides the single processes, the sonozone might result in higher bacterial inactivation rates [61]. Differences in the DBPs formation, comparing the sonozone and the single ozonation, have to be assessed. Toxicity evaluations are mandatory, being a serious matter of concern when involving chemical reactions for the disinfection of the WW. Anyway, limited studies are reported in literature regarding the sonozone application for the urban WW reuse.

2.4 Legislative frameworks

Countries and international organizations commonly define specific guidelines and regulations to classify and standardize the number of parameters (physical, chemical, and biological) needed for wastewater characterization. Different criteria may be required depending on the discharge location and for the WWTP size, seen as number of P.E. [62]. Moreover, a survey of the available worldwide regulations/guidelines for RWW for agricultural reuses was conducted: as the related legislative framework is constantly evolving, a highly inhomogeneous situation was highlighted. Regulations may differ concerning targeted parameters and threshold limits, e.g., coliforms can be enforced in different countries as total coliforms, E. Coli, or thermal coliforms [63]. In addition, thresholds may differ according to crop types, being generally stricter for contact irrigation of crops intended for raw human consumption. Among all the defined ones from Table 2-1, a small number of them is regularly monitored.

The most important documents considered will be explained in the following sub-sections.

2.4.1 Directive 91/271/EEC

The first council directive shared through the “Official Journal of the European Communities” was the 91/271/EEC of 21 May 1991 “concerning urban waste water treatment” [62]. The “Requirements for discharges from urban wastewater treatment plants” as a threshold, or a percentage of reduction, in the effluent concentration compared to the influent characteristics are reported. The WW quality is characterized with the organic matter content, BOD_5 (without nitrification) and COD, the TSS, and the nutrients, through TP and TN. To be accepted for a safe discharge, an effluent parameter must show a concentration below the given limits or a reduction percentage, compared to the influent value, higher than the one established by the Directive. Moreover, the European Directive suggests specific evaluation methodologies that have to be followed. Nevertheless, this Directive does not consider the heavy metals, pathogens and surfactants as mandatory quality controls, even though WW may include significant concentrations of these compounds. To sum up the information contained in the 91/271/EEC (Annex I, Table 1 and 2), the discharging requirements are reported in Table 2-3:

Table 2-3: 91/271/EEC defined parameters

Parameters and unit of measurement	Maximum concentration allowed	Minimum percentage of reduction
BOD_5 (mg/L) *	25	70 - 90
COD (mg/L)	125	75
TSS (mg/L)	35 (> 10.000 P.E.) 60 (2.000 - 10.000 P.E.)	90 (> 10.000 P.E.) 70 (2.000 - 10.000 P.E.)
TN (mg/L)	10 (> 100.000 P.E.) 15 (10.000 - 100.000 P.E.)	70 - 80
TP (mg/L)	1 (> 100.000 P.E.) 2 (10.000 - 100.000 P.E.)	80

* The BOD_5 can be replaced by the TOC if a relationship can be established between them.

2.4.2 Regulation (EU) 2020/741

The most recent “Regulation of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse” (EU 2020/741) is here considered [64]. The Directive defines four water quality classes for irrigation (Table 2-4) and is scheduled for adoption by all EU member states within the year 2022. This new EU regulation establish the requirements for each reuse class (A, B, C, D, with decreasing quality); the noncompliance of just a single parameter leads to demotion to a lower class. Remarkably, the four classes (Annex I, Section 1, Table 1) are:

A) All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw (all irrigation methods);
B) Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals (all irrigation methods);
C) Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals (drip irrigation or other irrigation method that avoids direct contact with the edible part of the crop);
D) Industrial, energy and seeded crops (all irrigation methods).

Table 2-4: RWW quality classes based on European Union Directive 2020/741

RWW quality class		**A**	**B**	**C**	**D**
Indicative technology target and unit of measurement		**Secondary treatment, filtration, and disinfection**	**Secondary treatment and disinfection**	**Secondary treatment and disinfection**	**Secondary treatment and disinfection**
Quality requirements and monitoring frequency***	E. coli (CFU/100 mL)	≤ 10 (once/week)	≤ 100 (once/week)	≤ 1.000 (twice/month)	≤ 10.000 (twice/month)
	BOD$_5$ (mg/L)	≤ 10 (once/week)	≤ 25 **	≤ 25 **	≤ 25 **
	COD (mg/L)	-	≤ 125 *	≤ 125 *	≤ 125 *
	TSS (mg/L)	≤ 10 (once/week)	≤ 35 (> 10.000 P.E.) ** ≤ 60 (2.000 - 10.000 P.E.) **	≤ 35 (> 10.000 P.E.) ** ≤ 60 (2.000 - 10.000 P.E.) **	≤ 35 (> 10.000 P.E.) ** ≤ 60 (2.000 - 10.000 P.E.) **
	Turbidity (NTU)	≤ 5 (continuous)	-	-	-
	Other	Legionella spp. (twice/month): < 1.000 CFU/L where there is aerosolization risk Intestinal nematodes (helminth eggs) (twice/month or as determined by operators according to the number of eggs in influent wastewater): ≤ 1 egg/L for pasture or forage irrigation			

* Parameter not mentioned according to EU regulation 2020/741; limits set as reported in Directive 91/271/EEC [62]

** In accordance with Directive 91/271/EEC (Annex I, Table 1 and Section D).

***Monitoring frequencies: minimum annual samples determined according to WWTP size.

- 2.000 - 9.999 P.E.: 12 samples during the first year. 4 samples in subsequent years, if effluent compliance to the Directive during the year is proven, otherwise 12 samples;
- 10.000 - 49.999 P.E.: 12 samples/year;
- > 50.000 P.E.: 24 samples/year.

The new EU regulation, beside establishing minimum requirements for RWW, defines (Annex II) site-specific risk management approaches, specifying standards for data transparency. The entire cycle of wastewater reclamation (from sewers to WWTPs, to final reuse) is considered in a risk assessment approach, including analysis of environmental and health hazards, monitoring strategies, and protocols for emergency situations.

For consistency of comparison, a few assumptions were made herein:

1) in the absence of aerosols formation during irrigation (e.g., when using sprinklers), Legionella spp. was omitted from subsequent considerations;

2) the 5-days Biochemical oxygen demand (BOD$_5$) and total suspended solids (TSS) thresholds for classes B, C and D were reported in accordance with Directive 91/271/EEC;

3) chemical oxygen demand (COD), not mentioned in EU Regulation 2020/741, was included in the analysis, with limits set in Directive 91/271/EEC, since some existing regulations foresee COD rather than BOD$_5$ as relevant parameter;

4) turbidity was considered a required parameter only for the highest quality RWW (class A).

5) Exclusively for the class A, the indicator microorganisms have to show a minimum logarithmic reduction in order to validate the monitoring (Annex I, Section 2, Table 4).

Even though the Directive 2020/741 specifies all the mandatory compounds for a WW safe reuse, the N and P compounds are not mentioned at all. Then, nutrients and additional hazardous compounds are explained through the detailed study of the Italian national legislation.

2.4.3 Ministerial Decree 185/2003

The Italian guidelines towards a WW agricultural reuse are one of the strictest and most complete in the world [65]. The main regulatory document for the WW reuse is the Ministerial Decree of 12 June 2003, number 185 (185/2003 – “Regolamento recante norme tecniche per il riutilizzo delle acque reflue”) [34]. Several parameters are defined within it. Remarkably, the Directive 91/271/EEC and the Regulation 2020/741 are not exhaustive managing the range of possible pollutants produced through the AOPs disinfection process, while considering the adoption of classical secondary and tertiary WWTPs treatments. So, additional monitored compounds are indispensable in these cases. Analyzing in depth the Decree 185/2003, 55 different parameters are introduced, including their thresholds. However, only few of them will be assessed during the following consideration for an agricultural reuse, and subsequently presented (Table 2-5).

Table 2-5: 185/2003 considered limit values for agricultural reuse

Parameters and unit of measurement	RWW threshold
pH (-)	6 - 9,5
TSS (mg/L)	10
BOD_5 (mg/L)	20
COD (mg/L)	100
TN (mg/L)	15
Ammonia (mg/L)	2
TP (mg/L)	2
Total aldehydes (mg/L)	0,5
Total surfactants (mg/L)	0,5
E. Coli (CFU/100 mL)	100
Salmonella (CFU/100 mL)	not present

The reported limits for TN and TP could be considered, respectively, increased up to 35 mg/L and 10 mg/L in case of treated WW reuse not on nitrate-vulnerable soils [52]. Howsoever, the soil properties have to be previously studied.

2.5 Chapter Conclusions

An innovative WW treatment will be proposed with the current study in order to present new solutions for the water scarcity. The climate change effects and the population growth made wide areas of the world suffering from unavailability of clean and sanitized water, requiring advanced water-recovering processes. The IWC, being a small part of the bigger natural water cycle, is helpful to reestablish a balance in the environment after the anthropic exploitation of the water resources. Towards the current project's purpose, the interest mostly relies on the primary effluent (PEff) from the urban WWTPs. This is the WW, treated exclusively through the pretreatments and the primary clarifier, sampled before the entrance of the ACS process. The meaningfulness about this design choice is that only part of the solids was removed before the sample collection, while all the further remarkable compounds, including nutrients, are not. Those parameters could be classified as shown in Table 2-1 and the given limits are reported in the national and international regulations. The main target for the WW reclamation is defined as the agricultural sector, not excluding a future application to the industrial or civil necessities. The technology may be successfully introduced in dry areas, reducing the freshwater exploitation. The fertigation practice is the ideal approach for the WW agricultural reuse. The recovery of water and nutrients contributes to the circular economy perspective through the reduced need for both chemical fertilizers and freshwater supply in the agricultural sector. Moreover, the above-mentioned IWC qualitative closing prospective is supported. To guarantee whether the PEff reuse is feasible, maintaining the hazardous compounds under the legislative given thresholds, an additional disinfection is mandatory in any case. However, the preservation of nutrients (N and P) has to be guaranteed. A safe and valuable reclamation of the WW is indispensable. The implementation, initially at the laboratory scale, of two AOPs is proposed. The sonozone process is the hypothesized solution for the RWW agricultural reuse, as it demonstrated in literature the capacity of remove the hazardous compounds from the WW yet still maintaining the nutrients unaltered. Anyway, limited experiences were reported on combined US and O_3 applications on PEff treatment. The study is performed under different conditions to evaluate the system efficiency. Looking at a global perspective, regulations or guidelines adopted to assess the WW flows could be even stricter compared to 2020/741, adding a larger number of requirements or stricter thresholds. The decision made was to perform the WW reuse exclusively on crops described as classes B, C or D from Table 2-4. Furthermore, the nutrient limit values and well-known by-products and harmful compounds will be added from the Italian 185/2003 decree. The following Table 2-6 is beneficial to sum up the parameters utilized as a reference in the following:

Table 2-6: RWW thresholds references for the current study

Parameters and unit of measurement	RWW threshold
pH (-)	6 - 9,5
TSS (mg/L)	10
BOD_5 (mg/L)	20
COD (mg/L)	100
TN (mg/L)	15 (35) *
Ammonia (mg/L)	2
TP (mg/L)	2 (10) *
Total aldehydes (mg/L)	0,5
Total surfactants (mg/L)	0,5
E. Coli (CFU/100 mL)	100
Salmonella (CFU/100 mL)	not present

*: values inside the parentheses when not nitrate-vulnerable soils are considered.

The turbidity evaluation is not mandatory. In the absence of aerosols formation during irrigation (e.g., when using sprinklers), Legionella spp. was omitted from subsequent considerations. Furthermore, a basic heavy metals assessment was conducted for the extent of comparing their PEff concentrations with the legislation thresholds. The evaluation of the soil sensibility towards the nitrates could allow to select the higher thresholds for TN and TP. In addition, an in-depth analysis will be performed for the microbiological pollutants. As well as *Salmonella*, seen as *Salmonella Enteritidis* (*S. Enteritidis*), and *E. Coli*, *Pseudomonas* spp. and *Enterococcus* spp. will be assessed.

3 MATERIALS AND METHODS

Nowadays, the adoption of sophisticated and precise tests for the assessment of the WW's main parameters is permitted by the available technologies. Those experimental tests are mandatory in order to evaluate the abatement efficiency occurred during the performed disinfection through the AOPs. The disinfection processes have to be accomplished starting from the smaller scale (laboratory-scale) before moving to bigger ones, such as pilot-scale and full-scale. Throughout the utilization of spectrophotometers, elemental analyzers or other complex instruments, each needed concentration may be calculated and compared to its raw value. Among AOPs, the combination of US and O_3 shown in literature promising opportunities within the RWW reuse. Volumes, concentrations and treatment duration have to be calibrated and, despite being well-known for the separate processes, carefully set for the sonozone combination and its replicability. Furthermore, the economic feasibility and the toxicity have to be reasonably demonstrated.

3.1 Analytical techniques

In this section, experimental instruments and adopted characterization processes will be defined.

3.1.1 Spectroscopy

Ultraviolet-Visible (UV-Vis) Spectroscopy is adopted in the monitoring of the absorbance (or transmittance) for WW samples in the laboratories. The UV-Vis range is covering the electromagnetic spectrum in the region from 100 to 700 nm. During a WW disinfection process, when color shifts from a darker to a lighter one, the difference in absorbance indicates the extent of organic abatement. Marked differences corresponds to higher absorbance outputs. The produced spectrum, single or multi wavelength, can be correlated to the identification of the compound concentrations. Being easier and cheaper compared the other instruments, a spectrophotometer was employed for the assessment of the concentration of several compounds of interest, such as TSS, COD, ammonia, nitrites, nitrates, TP, UV_{254}, O_3 concentration, surfactants and aldehydes. The methods to be followed for the characterization of the compounds are detailed in the manual "Standard methods for the examination of the water and wastewater" [66]. Aqueous tested samples are compared with a zero reference while subjected to a UV-Vis source. The comparison between the absorptions gives an indication regarding the organic abatement [67]. Cuvettes are used as containers during the measurements, made by glass, plastic or quartz. The

Hach-Lange DR5000 spectrophotometer was used. All the analytical measurements were performed in triplicate.

3.1.2 Elemental analyzer

TOC and TN were measured using a specific TOC/TN-TOCN-4110 Analyzer (Shimadzu) [68]. TOC methods use high temperature (typically 650 to 1200 °C) to convert organic carbon into carbon dioxide and measure it as fully oxidized. The method requires no reagents. The CO_2 may be purged from the sample via carrier gas to a nondispersive infrared analyzer (NDIR) or coulometric titrator. TOC is determined by measuring the non-purgeable organic carbon (NPOC), as the total carbon (TC) after inorganic carbon (IC) removal. Avoiding the IC removal, it is possible to obtain the TC, if desired. TOC combustion oxidation can easily be expanded to include the determination of TN.

3.1.3 Microbial analysis

Untreated (CTRL) and treated samples were diluted in Maximum Recovery Diluent (MRD) and decimal dilutions were analyzed using the drop plate technique [69] on Brain Heart Infusion agar (Oxoid). The plates were incubated for 24 h at 37 °C (*Enterococcus* spp., *E. coli* and *S. Enteritidis*) and at 30 °C (*Pseudomonas* spp.).

3.1.4 Other instruments

Instruments, such as High-Performance Liquid Chromatography - High Resolution Mass Spectrometry (HPLC-HRMS) and Gas Chromatography - Mass Spectrometry (GC-MS), may be applied towards the screening and measurement of several micropollutants contained in the WW flows and their qualitative assessments through the pre-injection of standard solution used as a reference of concentration [70], [71]. Samples were injected directly in the HPLC-HRMS instrument. The analysis was performed through a 1200 series capillary pumps and autosampler and a XBridge C18 3,5 µm (100 x 2,1 mm) column, using an Orbitrap Q Exactive MS. The identification of the substances was made by comparing MS spectra with the NIST14 library (when available) or by manual search on the ChemSpider database. As regarding the GC-MS system, samples (5 mL) were analyzed by exposing SPME fibers inside the vials, and using the Agilent 5975 MSD, with standard EI source, positive ions (EI+). The GC column was a Varian CP Select 624, 60 m x 320 µm x 1.8 µm, with a 1,2 mL/min of helium flow. The oven program was set at 40 °C for 1 min, then 10 °C/min to 250 °C for 2 min.

A Horiba Laser scattering particle size distribution analyzer LA-950 (measurement range: 1 nm to 5000 nm) was used to get the dimensional distribution of TSS present in PEff samples. The instrument employs laser diffraction as the optical system to obtain particle size distribution (expressed as volume percentage).

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. This technique works on the principle that all elements emit energy only at specific wavelengths which is related to their atomic character. When the electrons come back from excited state to the ground state, a unique energy transfer occurs depending upon the electronic configuration of the orbitals of elements [72]. This analytical technique is useful for the determination of heavy metals in aqueous samples.

3.2 Sonozone equipment: laboratory scale

Initially, the processes were singularly tested and calibrated, setting the mechanisms and performances. The ultrasonic bench-top equipment was firstly tested in discontinuous mode and then with continuous cycles. Unlikely, the ozone bench-top system was designed for a semi-continuous application. Discontinuous or continuous is intended as the fluid inflow (air or water) ruled, respectively, by an automatic pump or a human repeatable operation. The peristaltic pump (Cellai Perinox SF3, 230 V, 80 W, 1,6 x 1,6 3R) was used to regulate the WW stream, with a flowrate ranging from 2,5 to 25 L/h.

3.2.1 Ultrasound processor: calibration and operating conditions

An ultrasonic processor UIP 250 (Hieschler Ultrasonics, Teltow, Germany), 250 W maximum power, equipped with a 24 kHz ultrasonic transducer having a BS24 titanium probe (22 mm diameter) was used (Fig. 3-a). The treatments were carried out at 20% of the maximum frequency amplitude, which corresponded to 4,8 kHz of working frequency and a mean absorbed power of 74 W (discontinuous mode) and 62 W (continuous mode).

In the calibration phase, a linear correlation between the sonication time (5 - 600 s) and the measured UV absorbance, at a fixed wavelength of 355 nm of a standardize KI solution, was observed for different amplitudes (20%, 40%, 60%, 80% and 100%) of the US device. A similar linear correlation emerged between sonication time and pump flows, useful to select the 3 different sonication continuous retention times (RT). The chosen amplitude of 20% gave the highest linearity

($R^2 > 0,99$) among the various tested amplitude values, together with the reduced energy need. Low frequencies gave the possibility not to generate an uncontrolled heat increasing during the tests, thus reducing the power absorbed. The ultrasonic bench-top equipment was firstly tested in discontinuous mode, employing a movable glass reactor (200 mL). Then, once the calibration was complete, the metal reactor, 190 mL, was employed (Fig. 3-a). The reactor was equipped with a water-cooling mantel, to eventually keep the temperature at (20 ± 2) °C during the tests. The WW inflow inside this US reactor is from the bottom part. This value was selected to be consistent with the mean WW temperature in the selected WWTP.

Three sonication times were selected: 5, 10 and 30 s. These treatment times were chosen after energy considerations and the necessity of a subsequently combination with the ozonation process. Further details will be described in the chapter conclusions.

Figure 3-a: US laboratory reactor

3.2.2 Ozone generator: calibration and operating conditions

An ozone generator C-Lasky CL-010-DT (AirTree Ozone Technology Co., Sijhih City, Taiwan) was used to treat each WW sample (Fig 3-b). The ozone was produced through the generator from the air sucked from the external environment. Pressurized air or pure oxygen were not utilized as an inlet for the ozone production due to the higher costs, compared to their performances. During the treatment, ozone was introduced directly into the sample (2 - 2,5 L/min of air, 1 - 1,5 mg/L dissolved ozone) through a glass tube immersed in the sample for its entire height.

The ozonation system was assembled in semi-continuous mode (continuous in relation to the gas-ozone flow and discontinuous in relation to WW). The 60 mm diameter glass column (400 mm tall, 1 L max volume, 800 mL treated volume) contained a glass fine porous bottom diffuser to introduce ozone in the reactor, letting it dissolve while reaching the surface. Two washing bottles in series, containing a KI solution [73], trapped the residual gaseous O_3 at the outlet of the reactor. All ozonation tests were conducted introducing the volume of the sonicated PEff in the reactor under constant room temperature (20 ± 1 °C) and neutral pH (7,0 ± 0,5) conditions. Ozone gas production and flowrates were calibrated and monitored with respect to the iodometric method 2350-E [74]. Furthermore, a remarkable calibration needed for any ozone application was done through the Indigo colorimetric method (4500-O_3 B) to assess the maximum dissolved ozone concentration evaluation in tap water [66]. An average absorbed power of 135 W emerged from the laboratory tests.

Four ozonation times were selected: 60, 120, 300 and 600 s. These treatment times were chosen after energy considerations and in order to be applicable with the pilot scale reactor that will be additionally tested in the future. Further details will be described in the chapter conclusions.

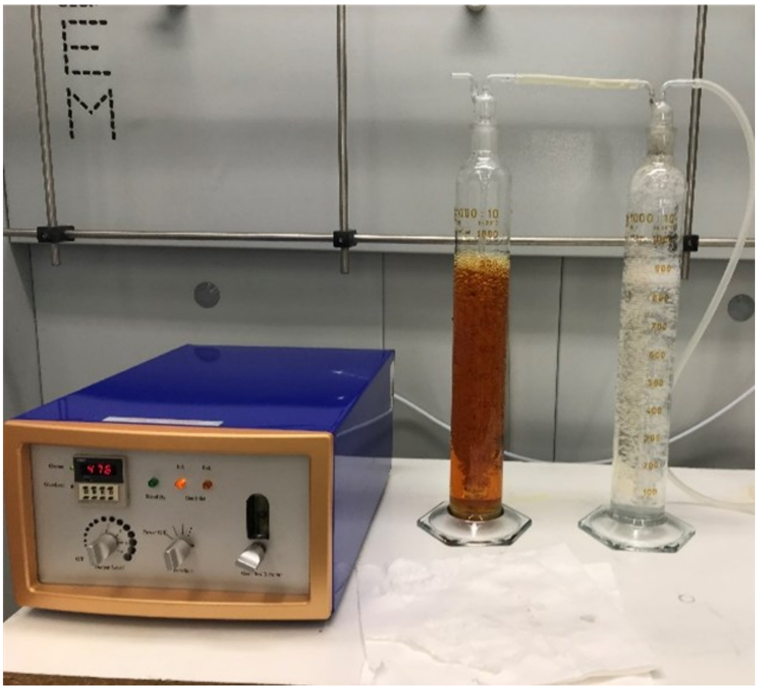

Figure 3-b: Laboratory scale ozone generator and reactors

3.2.3 Hybrid US + O3: design and treatment times

The laboratory sonozone setup was built as showed in Figure 3-c.

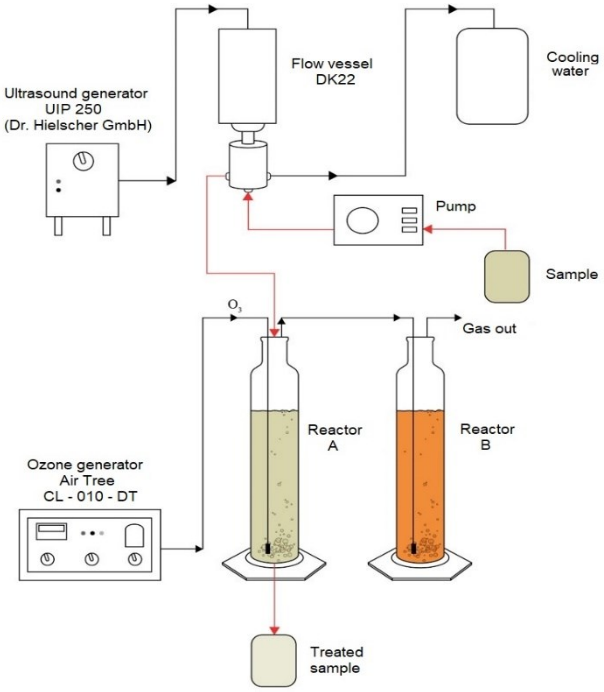

Figure 3-c: Combined laboratory sonozone system setup

The two branches were connected in series: a pump withdrew the sample and let it circulate in the sonication system. The retention time was controlled with the pump flow. The outflow from the ultrasound vessel was manually poured into the ozone tank where the gas is introduced through a bottom diffuser, for a known duration. The sample gathered from the ozonation vessel was the outcome of the overall treatment. Pre-sonicated WW samples were measured with the 2350-E method after the O_3 employment [74]. The residual ozone was captured in the reactor B (KI trap).
The combination of US and O_3 treatments are tested in the following reported in Table 3-1:

Table 3-1: Combination of US and O_3 tests - labels

US\O$_3$	**0 s**	**60 s**	**120 s**	**300 s**	**600 s**
0 s	US_0*O$_3$_0 (Not treated)	US_0*O$_3$_60 (only O$_3$)	US_0*O$_3$_120 (only O$_3$)	US_0*O$_3$_300 (only O$_3$)	O$_3$_600 (only O$_3$)
5 s	US_5*0$_3$_0 (only US)	US_5*O$_3$_60	US_5*O$_3$_120	US_5*O$_3$_300	US_5*O$_3$_600
10 s	US_10*0$_3$_0 (only US)	US_10*O$_3$_60	US_10*O$_3$_120	US_10*O$_3$_300	US_10*O$_3$_600
30 s	US_30*0$_3$_0 (only US)	US_30*O$_3$_60	US_30*O$_3$_120	US_30*O$_3$_300	US_30*O$_3$_600

In the following chapters, the combination will be labelled as in Table 3-1.

3.3 Sonozone equipment: pilot scale

The pilot scale laboratory tests should be intended as the passage from a semi-continuous laboratory scale (less than 1 L) to a continuous pilot plant (PP) (around 50 L), in order to be able to treat 0,5 - 1 m^3/day, running continuously, as a further step of the proposed agricultural reuse (Fig. 3-d).

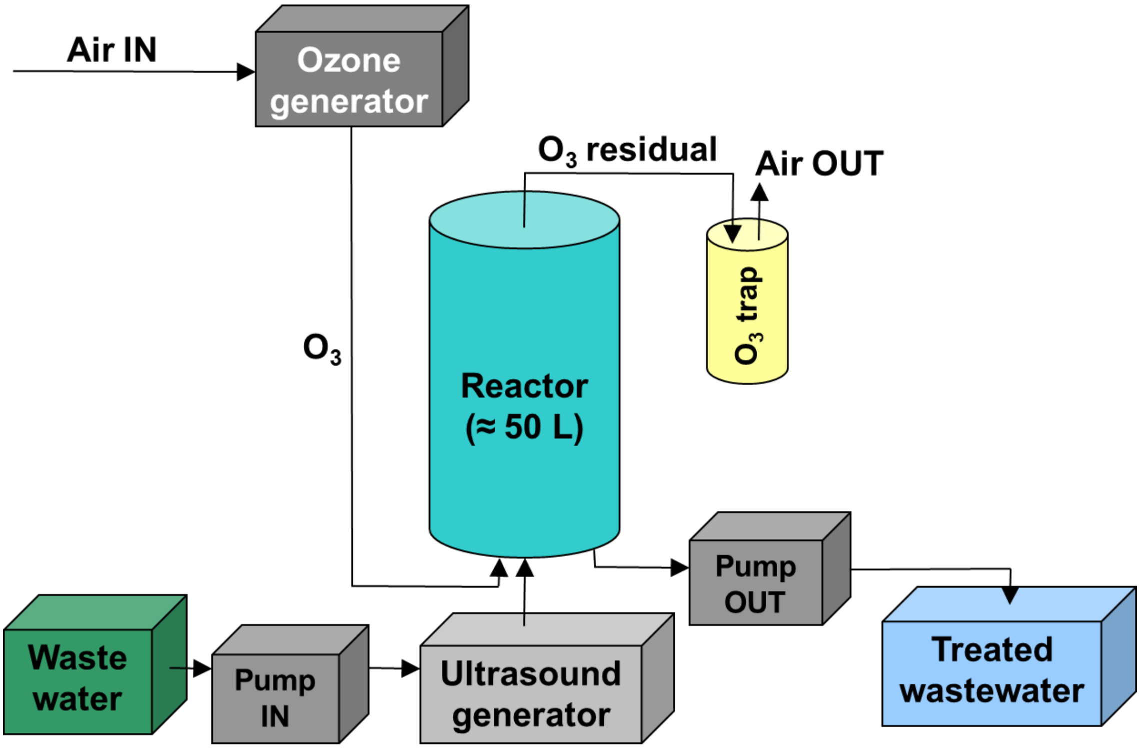

Figure 3-d: Pilot plant setup

Herein, the WW sample is pumped inside the PP with a pre-determined flow rate. Firstly, the sample is subjected to the US action, adopting the same US reactor described in the laboratory scale section. Then the pretreated WW is injected into the bigger reactor (around 50 L) and processed through the ozone flow. The WW and the ozone are both entering from the central bottom part of the main reactor, through different inlets. The process duration is based on the pump flow and previously fixed. As any similar works was found in literature, the treatment duration was based on the filling time of the internal column; based on it, the total process time will be calculated. Moreover, the chosen ozone retention time could not be excessively short due to the limited capacity of the ozone to be dissolved and the higher turbulences developed. In the same way, it could not be excessive to allow treating the 0,5 - 1 m^3/day. So, the decision was to keep the duration of the flow in the internal cylinder approximatively around 10 minutes. Then, while saturated, from the upper part of the reactor the residual undissolved ozone was collected inside a KI trap and the fully treated WW was gathered in a reactor. An initial calibration for the dissolved ozone was performed and all the instruments employed were the same as done in the laboratory tests (pump, US generator and ozonator). The materials, hydrodynamic properties and ozone dissolution rates were assessed as follows.

3.3.1 Materials

Dimension are referred to Fig. 3-e and 3-f, the components to Fig. 3-g.

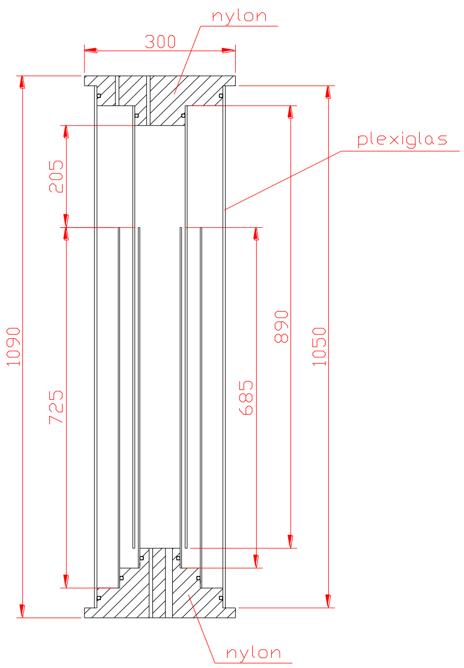

Figure 3-e: Pilot plant column heights

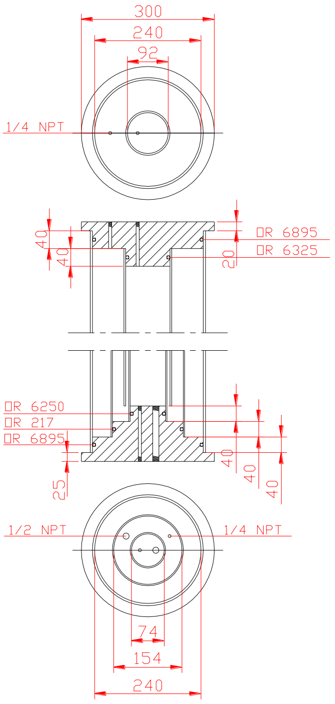

Figure 3-f: Pilot plant columns diameters

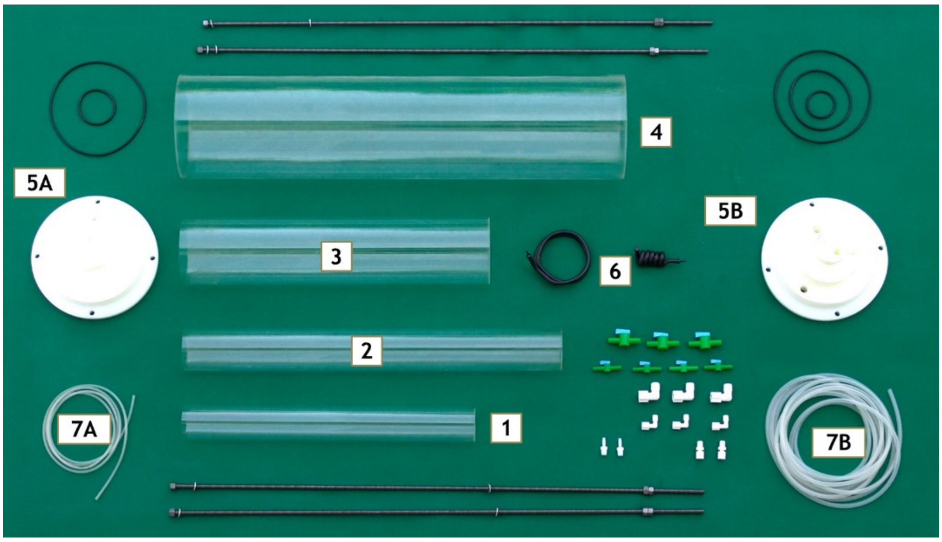

Figure 3-g: Pilot plant components

The main reactor, around 50 L of internal volume and 1 m height, was built with Plexiglas cylinders of different height, nylon supports at the top and at the bottom where the columns are locked in. The height of the column was designed in order to let the ozone act for a longer time on the organic targets. Iron screwable bars, circular seals and plastic taps were needed. The geometrical characteristics of the columns are:

1: 64,5 cm effective height and 74 cm diameter, approximatively 3 L volume. Locked on the bottom support;

2: 85 cm effective height and 92 cm diameter. Insert to maintain the pressure in the internal column. Locked on the upper support;

3: 68,5 cm effective height and 154 cm diameter, approximatively 10 L volume. Locked on the bottom support;

4: 97 cm effective height and 240 cm diameter, approximatively 33 L volume. Locked on both supports.

The reactor was arranged as described, with concentric cylinders locked on the nylon supports (upper support 5A, bottom support 5B). Apposite holes were created in the supports in order to have inlet and outlet spots for the gaseous and liquid flows. The chosen materials for the structure are well-known ozone-resistant materials. They could excellently resist to a solution rich in ozone. A flexible pierced black pipe was used as an ozone diffuser (6). Furthermore, ozone compatible silicon pipes of different lengths were used (7A). Silicon tubes were equally employed for the WW flow, only having a larger diameter (7B).

3.3.2 Operations

The WW circulates into the reactor (Fig. 3-h) through a one-inch hole in the column “a”. The pumped flow reaches the upper central border of the column “a” and the stream flows down to column “b”, executing the same passages until column “c”. At the bottom of column “b” and “c” two different one-inch holes may be used for the WW outfall. The ozone is introduced through an air diffuser pipe from the bottom of both columns “a” and “b”. The accomplishing of the dissolution of the whole produced ozone into the WW sample is the main target of this kind of flowing system. However, a partial amount of ozone will remain undissolved, being expelled through the hole placed in the upper part of the column “c” and collected in a KI trap. Another emergency upper exit hole is located in the upper ceiling of the column “b”, usually sealed.

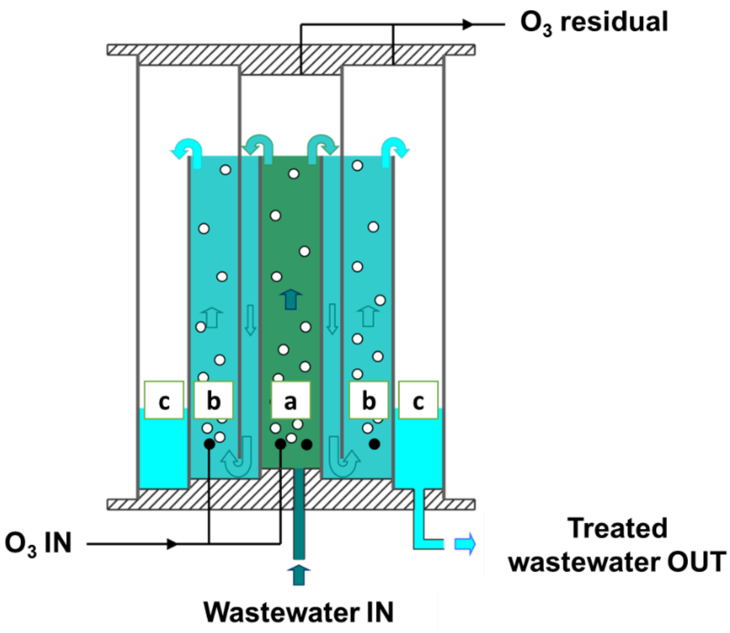

Figure 3-h: Pilot plant flow scheme

To sum up, the WW is injected into the reactor only from column "a"; unlikely, the ozone is split in two flows and introduced from column "a" and "b" continuously. Generally, the purpose of the reactor is to let the ozone and the WW to stay in contact as much as possible, then the preferred exit for the WW is at the bottom of column "c". The ideal movement of the WW and ozone inside the reactor is as a plug flow. Diameters and heights of the columns are reported in Fig. 3-e and 3-f.

3.4 Microbial in-depth assessment

The bench-top instruments utilized during the microbial in-depth study were the same US and O_3 above-described. Air flows and frequencies were maintained the same, however the treatment times were changed, based on the indicator pathogen. For each US test, 200 mL of spiked WW were placed under the sonication probe, discontinuity in a glass sterilized reactor, for 5, 10, 30, 60, 90, 180, 300, 450, or 600 s in a bath with water recirculation to maintain the temperature at about 23 °C. The samples were named US_5, US_10, US_30, US_60, US_90, US_180, US_300, US_450, US_600, where the number corresponded to the time (s) of ultrasound treatment. Ozonation was carried out for 30, 60, 120, 240, 360, 480, 600, 750, and 900 s; samples were labeled as O_3_30, O_3_60, O_3_120, O_3_240, O_3_360, O_3_480, O_3_600, O_3_750, O_3_900, where the number refers to the duration time (s) of the treatment. In the last step, spiked WWs were treated with US and O_3 applied one after the other. Four levels of treatment with US (30, 90, 300 and 600 s) and two of O_3 (60 and 120 s for *Pseudomonas* spp., 30 and 60 s for *Enterococcus* spp., 15 and 30 s for *E. coli*, and 30 and 45 s for *S.* Enteritidis) were applied, resulting in eight different treatments for each pool. Treatments were labeled using the name of the US treatment and the ozone treatment separated by an asterisk. Accordingly, treatment of US for 30 s followed by ozone for 60 s was named US_30*O_3_60, and so on.

The WW samples, before each test, were filtered with gauze to remove coarse suspended particles, and then sterilized at 121 °C for 15 min. *Pseudomonas* spp., *E. Coli*, *S*. Enteritidis and *Enterococcus* spp., previously isolated from urban WW, were used to spike the sterilized samples. Before each test, strains were separately grown overnight in 33 mL of BHI at 30 °C (*Pseudomonas*) or 37 °C (*E. coli*, *Enterococcus* and *Salmonella*). After the laboratory experiments, the treated samples were plated on BHI agar (Oxoid), the microbial cell count was executed through the drop-plate method [69]. Hence, the plates were incubated for 24 h at a fixed temperature (37 °C *Enterococcus* spp., *E. coli* and *S*. Enteritidis and 30 °C *Pseudomonas* spp.). All trials were carried out in at least two biological replicates.

Moreover, for samples treated only with ozone, viable counts versus exposure time were modelled according to the Weibull + Tail distribution shown in *Eq. 1* using the Excel add-in GInaFiT [75]:

$$N = log_{10}\left[\left(10^{log_{10}(N_0)} - 10^{log_{10}(N_{res})}\right) \times 10^{\left(-\left(\frac{t}{\delta}\right)^{p}\right)} + 10^{log_{10}(N_{res})}\right] \qquad \textit{(Eq. 1)}$$

where N represents the microbial concentration (CFU/mL); N_0 is the initial concentration (CFU/mL); N_{res} the residual microbial population (CFU/mL); t expresses the time (s); p indicates a shape parameter (> 1 for concave curves or < 1 for convex curves) and δ serve as time required to obtain the first decimal reduction (s). Moreover, the model provides the 4D value, i.e., the time required to obtain 4 logarithmic reductions of N_0.

3.5 Economic assessment

The achievement of a reliable technology, suitable for any industrial application, have to possess two main characteristics: technical feasibility and economic advantages. In order to assess the benefits of the sonozone process, an economic interpretation is given.

3.5.1 General calculation

Starting from the single consumptions, the absorbed energy varies among the US and O_3 utilization, likewise applying different treatment times. The average requirement of energy (E, Wh) was obtained through *Eq. 2*:

$$E = P\,t \qquad \textit{(Eq. 2)}$$

The parameter t indicates the time, expressed in hours (h).

All the time, expressed in seconds, are converted in hours. Moreover, the data elaboration has to be referred to the single volume (V) unit (m^3).

In general, a basic comparison between the enhanced removal rate of the sonozone process and its augment in the energy consumption has been made. Considering an Italian case study, the standard purchasing prize for a kWh ($C_€$) is set at 23,4 c€/kWh [76]. To estimate the annual energy consumption cost, the processes were considered as continuously running.

3.5.2 Feasibility towards a microbial recovery: the in-depth method

Herein, an in-depth method for the energy and cost assessment towards a microbial recovery will be presented. The energy savings and the cost reduction are related and expresses as follows.

3.5.2.1 Energy savings:

The combined US and O_3 measured energy consumptions per cubic meter ($E_{US \cdot O_3}$) are evaluated through the *Eq. 3*:

$$E_{US*O_3} = E_{US} + E_{O_3} = \frac{P_{US}\, t_{US}}{V} + \frac{P_{O_3}\, t_{O_3}}{V} \qquad (Eq.\ 3)$$

Herein, P_{US} and t_{US} indicate respectively the absorbed power of the ultrasound generator (kW) for a given time (h). In the same way, P_{O_3} is the absorbed power of the ozone generator during the t_{O_3} application. V (m^3) is the maximum volume treated in one hour.

Then, the identified energy consumption per meter cube ${E_{O_3}}^*$ will be defined as follows:

$${E_{O_3}}^* = E_{O_3} + \Delta E_{O_3} \qquad (Eq.\ 4)$$

In particular,

$$E_{O_3} = \frac{P_{O_3}\, t_{O_3}}{V} \qquad (Eq.\ 5)$$

and

$$\Delta E_{O_3} = \frac{P_{O_3}\, \Delta t_{O_3}}{V} \qquad (Eq.\ 6)$$

where Δt_{O_3} is defined as the additional time needed, during an only ozone application, to reach a logarithmic reduction equal to the one obtained after the combined US and O_3 process. Δt_{O_3} was calculated though the identified abatement equation (*Eq. 1*).

For the extent of obtaining the above-mentioned ${E_{O_3}}^*$, the ΔE_{O_3} have to be summed with the only O_3 consumption E_{O_3} (*Eq. 4*).

To assess whether the combined process will be economically convenient, the sonozone energy savings ($E_{s\%}$) were calculated as follows:

$$E_{s\%} = 1 - \frac{E_{US*O_3}}{E_{US} + {E_{O_3}}^*} \qquad (Eq.\ 7)$$

When the $\mathrm{E}_{s\%}$ resulted to be positive, an economic advantage is obtained in a combined application of the processes; otherwise, the performances of the separate processes had a better economic feasibility.

3.5.2.2 Cost reduction:

The cost of the combined sonozone ($\mathrm{M}_{\mathrm{US}*\mathrm{O}_3}$), single US ($\mathrm{M}_{\mathrm{US}}$) and single identified O3 ($\mathrm{M}_{\mathrm{O}_3{}^*}$) treatments were calculated, respectively, through *Eq. 8*, *Eq. 9* and *Eq. 10*:

$$M_{US*O_3} = E_{US*O_3} * C_€ \qquad (Eq.\ 8)$$

$$M_{US} = E_{US} * C_€ \qquad (Eq.\ 9)$$

$$M_{O_3{}^*} = E_{O_3}{}^* * C_€ \qquad (Eq.\ 10)$$

The total savings are defined through *Eq. 11*:

$$\Delta M = \left(M_{O_3{}^*} + M_{US}\right) - M_{US*O_3} \qquad (Eq.\ 11)$$

The costs are all expressed in €/m^3.

3.6 Ecotoxicological evaluation

Ecotoxicological and biomonitoring approaches are fundamental to assess both effluent and soil characteristics during fertigation, evaluating practice safety under all aspects (environment, workers, consumers) [77]. This is particularly important in high-quality crop applications or crops that have to be eaten raw when the edible parts come in direct contact with fertigation water [78]. Different methods can be used to biomonitor wastewater effluents, including traditional acute tests (Fish, Daphnia magna and Algae) or in vitro methods (inhibition of enzyme carbonic anhydrase). Soil quality can be assessed by acute and chronic tests, as well as through biomarker analysis [77]. Hazardous compounds need to be carefully monitored when applying RWW strategies: a quantitative chemical risk assessment was proposed as a robust framework to quantify infection/illness probability, allowing to determine the reuse approaches [79].

The chemical risk assessment was performed in-silico, through the VEGA software developed by the "Istituto di Ricerche Farmacologiche Mario Negri" (IRFMN). The software allows to apply several models, decided by the user, where the chemical compounds are imported and elaborated, returning as an output the complete reports containing the predictions [80]. The chemical information was downloaded from the PubChem online collection and uploaded into the VEGA library. Then, the models for the calculation have to be selected. The available ones are the: Toxicological, Eco-toxicological, Environmental and Physical-Chemical. The output of each assessment is linked to a reliability given by the software. For the further analysis, exclusively the moderate and good reliability outputs will be considered. The chosen models are:

Ecotoxicological - Fish acute (Lethal Concentration causing the death of the 50% of the population - LC50), Fish chronic (highest value for No Observed Effect Concentration - NOEC), Daphnia Magna acute (Effective Concentration causing a certain effect for the 50% of the population - EC50), Daphnia Magna chronic (NOEC), Algae acute (EC50) and Algae chronic (NOEC);

Environmental – Persistence in soils and water (quantitative IRFMN model);

Physical-Chemical – Water solubility.

Different ecotoxicological test were performed in order to have a reference for different organisms, like vertebrates (fish), invertebrates (Daphnia Magna) and plants (algae). Daphnia Magna and algae regularly possess a higher sensitivity towards the acute and chronic assessment than the fish [81]. Related to the toxicities, the persistence in soils and water could enrich the value. Often, toxic compounds may be long-lasting in different environments, provoking deficiencies even at a very low concentration [82]. Being in contact with soil and water, the human nourishment could be exposed to the pollution of persistent compounds. Some compounds could be easily biodegradable, half-life (HL) reduction < 30 days, however further chemicals may last for years or decades [83]. Moreover, the solubility in water indicates the amount of that specific substance that can dissolve in water. Higher capacity of dissolution in water means that the compound would be rapidly absorbed by the organisms, whereas an almost insoluble compound may settle or filter in the water layers.

The compounds that will be presented in the ecotoxicological assessment are hazardous volatile and persistent chemicals, such as aldehydes, alcohols, pesticides, health care and cleaning products, pharmaceuticals and more.

3.7 Experimental repeatability

The entire laboratory experience was based on specific tests repeated multiple times, executed within 48 h after the sampling or the treatment. As a consolidated practice in the field, the sample was tested two or three times (depending on the available volume) using the same characterization method. Additional samples were taken at different times, assuring that the environmental conditions were similar to the previous samples (i.e. weather conditions, time of the day, external interferences in the WWTP inflow, etc.). Generally, the results are evaluated and reported in figures as mean value (the bigger bar) ± standard deviation (the error bar). The reproducibility of the tests, meant as the ability to obtain similar results when conducting the experiment in analogous conditions, and the repeatability, the replication of an experiment based on the described methods, depend on the WW inflow [84] considered as a sample (urban and industrial may vary different logarithmic units of concentrations). The most significant parameters that must constantly be under control during the experimental evaluation were the liquid temperature, the ozonation time, the ozone airflow, the liquid pumped flow, and the ultrasonic amplitude. pH, despite being a major parameter, was considered constant in an urban WW sample.

3.8 Chapter conclusions

In order to define the sonozone efficiency, a series of physico-chemical and biological parameters were analyzed previously to the beginning of the processes, comparing them to the post treatment ones. For most cases, the experimental assessment relies on several standardized spectrophotometric measurements. HPLC-HRMS and GC-MS tests, microbial strain count on plates and elemental analysis are additional employed characterization processes. Before the examination, each sample was filtered through a 0,45 µm filter to prevent residual particulate interferences during the successive measurements (except for the TSS assessment). The results, that will be shown in the next chapter, represent the dissolved portion of the compound remained after filtration, such as dissolved COD, dissolved TN, and so on. Eventually, dilutions were required.

US treatments were carried out at 4,8 kHz of working frequency. During the treatment, ozone was introduced directly into the reactor, containing the sample. The ozone generator worked at 2 - 2,5 L/min of air, corresponding to a 1 - 1,5 mg/L of dissolved ozone. Three sonication and four ozone durations were selected. The decision to maintain a US duration under the 30 s was related to three main aspects: the energy consumption, the heating of the WW and the difficulties to combine the US with the following ozone treatment. Longer treatments would have caused an increase in the liquid temperature even above 50 °C, not being directly exposable to an ozone process. The possible remedies are an introduction of a cooling flow (possible in the US reactor chamber), involving unavoidable considerable energy consumptions, or letting the sample rest at a room temperature and cool down slowly, entailing an impossibility to perform a continuous flow between the combined treatments. These considerations have been made since the beginning of the hybrid US and O_3 process. O_3 is a strong oxidizer that has great disinfectant activity, which finds wide application in many fields including medical, agricultural, marine, and food to inactivate freely suspended and surface-attached microorganisms [85], [86]. However, the maintenance of a controlled temperature and pH is mandatory. The main consideration is, while working with a PEff from an urban WWTP, that the range of maximum and minimum temperature and pH along the seasons is limited. Despite having registered an external air temperature of - 2,5 °C, the WW showed a minimum value around 11 °C [87]. So, the range of thermal differences between the cold season (10 - 14 °C) and the summer (18 - 22 °C) kept close to 10 °C. Values can slightly change in far-located WWTPs, in relation of the P.E. number. In accordance, pH is essentially constant during the year. Consequently, the experiments were performed without the requirement of cooling processes or acidification, avoiding time-consuming pre-processes and additional monetary efforts. Furthermore, several recent literature studies have reported ozone applications, especially during hybrid AOPs, associated with more than 10 minutes durations [88]. However, the ozone concentrations (30 - 1000 mg/L) are not comparable with the present. Again, similar concentrations were considered for the pharmaceutical mineralization from WW flows [89] and for the degradation of the organics [90]. Performing the ozonation alone for more than 10 minutes would not allow to treat 0,5 - 1 m^3/day through the PP reactor, resulting in not being fruitful for the project. Thus, the four tested ozone duration were selected as shorter or equal than 10 minutes.

The hybrid treatment consists in the connection in series of US and O_3. Furthermore, the laboratory-scale hybrid process was studied and the scaling up of the system was accomplished. The PP, intended as the transition from a semi-continuous laboratory scale (less than 1 L) to a continuous PP (around 50 L), was designed in order to be able to treat 0,5 - 1 m^3/day, running unstopped, as a part of the proposed agricultural reuse procedure. The PP structure is made by Plexiglass cylinders, nylon supports and silicon pipes that allowed to obtain a plug flow behavior during the pilot scale process.

In recent studies the sonozone treatment has demonstrated promising outcomes in WW treatment, showing a synergistic effect of US and O_3 thus enhancing the inactivation of coliforms, *Escherichia coli*, and enterococci of either method used alone [91], [92]. However, additional data are missing for other bacteria, e.g., *Salmonella* and *Pseudomonas*, which are frequently present in WW and are indicative of fecal contamination or potential regrowth [93], [94]. Furthermore, apart from general considerations, there are no detailed studies in which a careful assessment of the economic feasibility of the combined treatment has been made, which is a prerequisite for any full-scale application. The microbial in-depth assessment will quantitively evaluate the ozonation alone abatement rate and compare it to the sonozone efficiency. Moreover, a detailed economic assessment will be executed, balancing the single and hybrid removal rates with the related energy consumptions. This passage is mandatory for an utter understanding of the RWW fertigation safe reuse. The equations for the estimation of the energy savings and costs were reported.

Eco-toxicological, environmental and physico-chemical approaches are fundamental to assess both effluent and soil characteristics before the fertigation reuse. The microbial risk assessment was performed in-silico, through the VEGA software. The software allows to apply several models, suitable for the analysis of volatile and persistent compounds.

4 RESULTS AND DISCUSSION

The relevance of the hybrid US and O_3 technology is related to the strong cavitation effect that enhance the disinfectant action given by the ozone [61], even maintaining the nutrients after the oxidation [95]. It was demonstrated that the seasonal variation of the WWTP flows temperature was maintained under 10 °C of excursion and in a near-neutral range of pH. Not needing any adjustment in the PEff initial conditions before the experiments, the sonozone appeared to be efficient in the initial preparation, compared to other complex AOPs preparations. The contribution given by the possible reuse of the RWW in the agricultural sector may be remarkable towards the circular economy perspective, reducing both chemical fertilizer and freshwater supplies in the crop's lifecycle.

As a reminder, from here on the processes are labelled as US_5 for the US duration 5 s, O_3_60 for 60 s of ozone treatment, and so on. The combination of the series and the colors in the figures will give the sonozone combination, such as the US_5 bar considered in the O_3_60 s treatment have to be considered as the hybrid process developed by 5 s of US pretreatment and the 60 s of O_3 action, US_5*O_3_60.

4.1 Sonozone: laboratory-scale semi-continuous treatment

The wastewater collected for the laboratory tests was a primary effluent (the inflow of the biological treatment) from a municipal WWTP located in Udine, a city in the north-east of Italy (100.000 P.E.). All the bench-top experiments were accomplished in the 48 hours following the PEff sampling from the plant. Comparing the PEff with the WWTP inflow, a percentage of COD and TSS was removed through the settlement occurred during the initial sedimentation. To be more precise, an average abatement of the 20 - 25% of total COD and 50 - 60% of TSS from an urban WWTP was observe [52], [96].

The PEff characterization was reported in Table 4-1:

Table 4-1: PEff characterization

Parameters and unit of measurement	RWW threshold
pH (-)	7,10 ± 0,20
TSS (mg/L)	143,50 ± 2,10
TOC (mg/L) *	32,08 ± 1,85
COD (mg/L)	146,00 ± 4,12
TN (mg/L)	42,80 ± 2,34
Ammonia (mg/L)	30,50 ± 2,11
TP (mg/L)	5,30 ± 0,47
Formaldehydes (mg/L) **	0,33 ± 0,07
Anionic surfactants (mg/L) **	3,70 ± 0,07
E. Coli (CFU/100 mL)	$(1,40 \pm 0,27) \times 10^6$
Salmonella (CFU/100 mL)	Not present
Potassium (mg/L) ***	16,93 ± 0,25
Calcium (mg/L) ***	53,38 ± 0,79
Magnesium (mg/L) ***	20,69 ± 0,20
Enterococcus spp. (CFU/100 mL) ***	$(1,09 \pm 0,21) \times 10^6$

* TOC is adopted instead of BOD_5, the directive permits it when a demonstrated relation exists between these two parameters;

** Substituting the parameters of, respectively, Total Aldehydes and Total Surfactants;

*** Additional parameters not reported in any regulation.

It could be noted that the primary WW parameters concentrations (pH, TSS, COD, TOC, TN, TP, E. Coli) were slightly lower than the mean urban WWTPs concentrations reported in Table 2-1. The indicators of Total Aldehydes and Surfactants were substituted with Formaldehyde and Anionic Surfactants, respectively. The pathogens, abundantly measured in the wastewater, are particularly harmful for the human health. As an indicator of the microbiological water quality, *Enterococcus* spp. was also included. *Salmonella* was not traceable in the untreated WW. Moreover, potassium, calcium and magnesium were evaluated during the experimental campaign to validate the capacity of the ozone to not reduce meaningful nutrients for the plant growth. All tests were reproduced in double or triple.

4.1.1 Consumptions and concentrations

As a first step for the assessment of the hybrid US and O_3 performances, the percentage of ozone consumed during the whole treatment was carried out. Three sonication pretreatments (5, 10 and 30 s) and four different ozonation times (60, 120, 300 and 600 s) were tested. The ozone consumption rate is presented in Fig. 4-a. It is possible to clearly define an augment in the ozone consumption efficiency after the US pretreatment, compared to the sole ozone. Moreover, longer

US applications corresponded to higher percentages of consumed ozone. 30 s of US could lead to a mean 30% of additional consumed ozone.

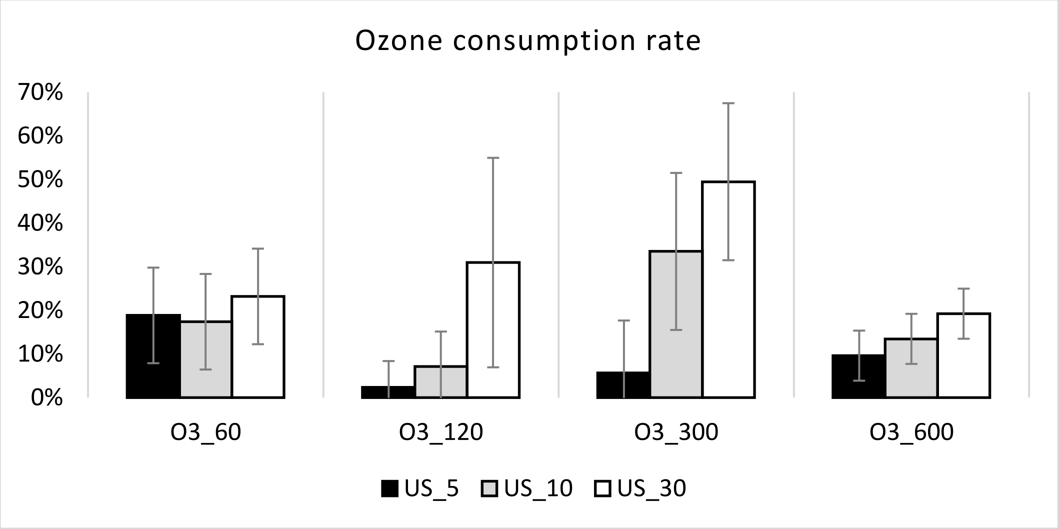

Figure 4-a: Ozone consumption rate

Then, the dissolved ozone (mg/L) was evaluated in order to set the level of concentration in pure water (method 4500-O_3 B, Standard methods). The results showed a mean concentration around 1,17 mg/L, stable along the treatment times (Fig. 4-b).

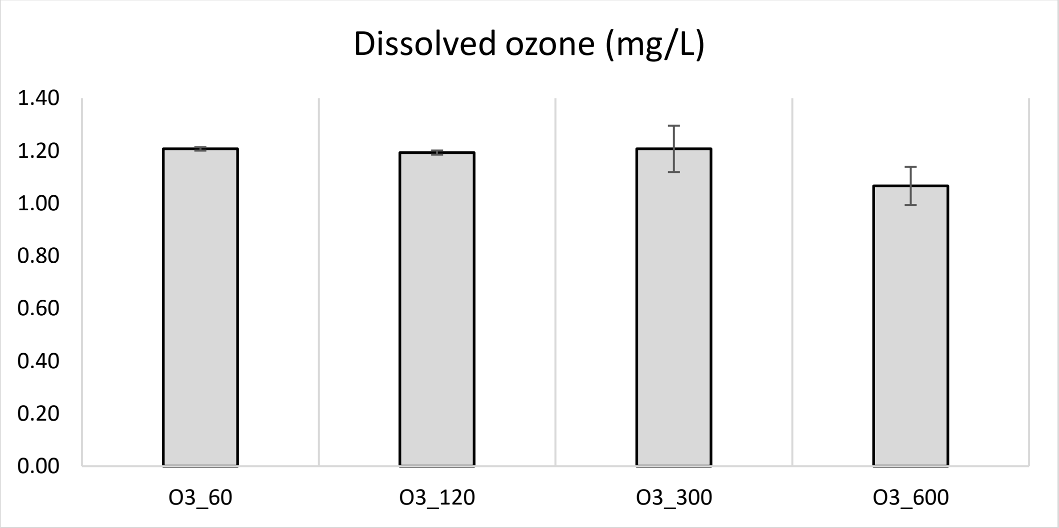

Figure 4-b: Dissolved ozone concentration in pure water

4.1.2 Effects on solids

From Figure 4-c it is possible to observe a significant drop in the concentration of TSS inside the WW sample after the treatment.

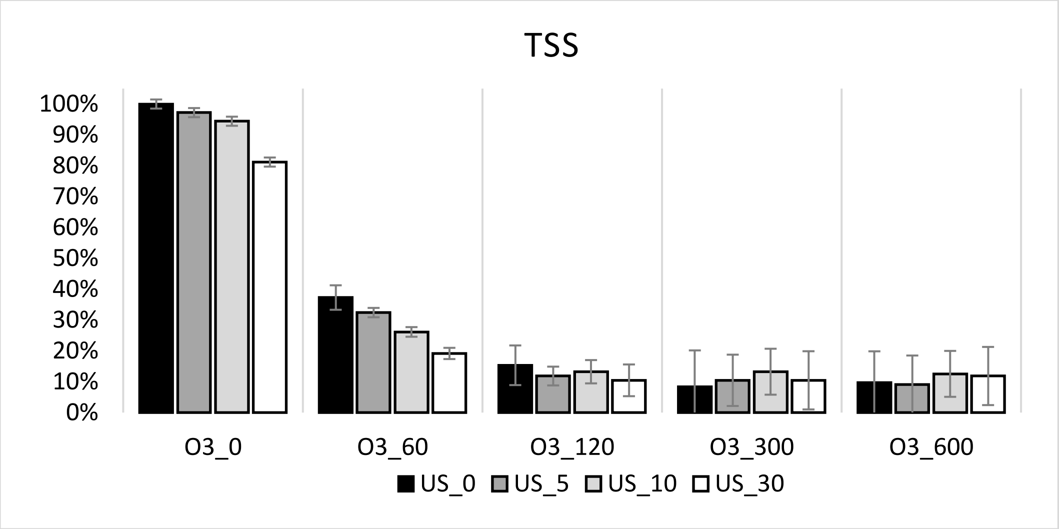

Figure 4-c: TSS residual percentage

The sole US action was mildly able to reduce the TSS content, less than the 20% for US_30 only. Percentages of abatement of more than the 60% were observed after 60 s of ozone, reaching the 85% with the maximum duration. For longer ozonation processes, the US pretreatment was not valuable due to the higher capacity of suspended solids removal given by the only ozone. However, from US_0*O_3_60 and US_30*O_3_60 was possible to clearly see a 15% of enhanced removal efficiency when the pretreatment is applied.

Moreover, an in-depth particle size analysis was performed in order to define the cavitation capacity in the reduction of the suspended solids diameter.

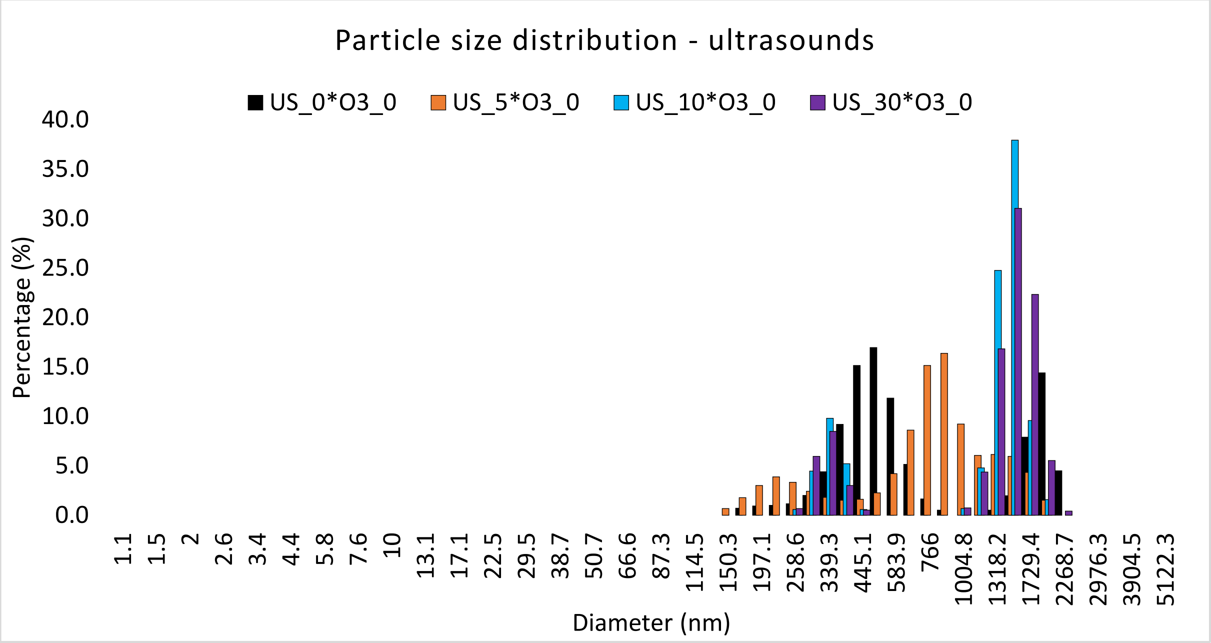

Figure 4-d: Ultrasounds particle size distribution

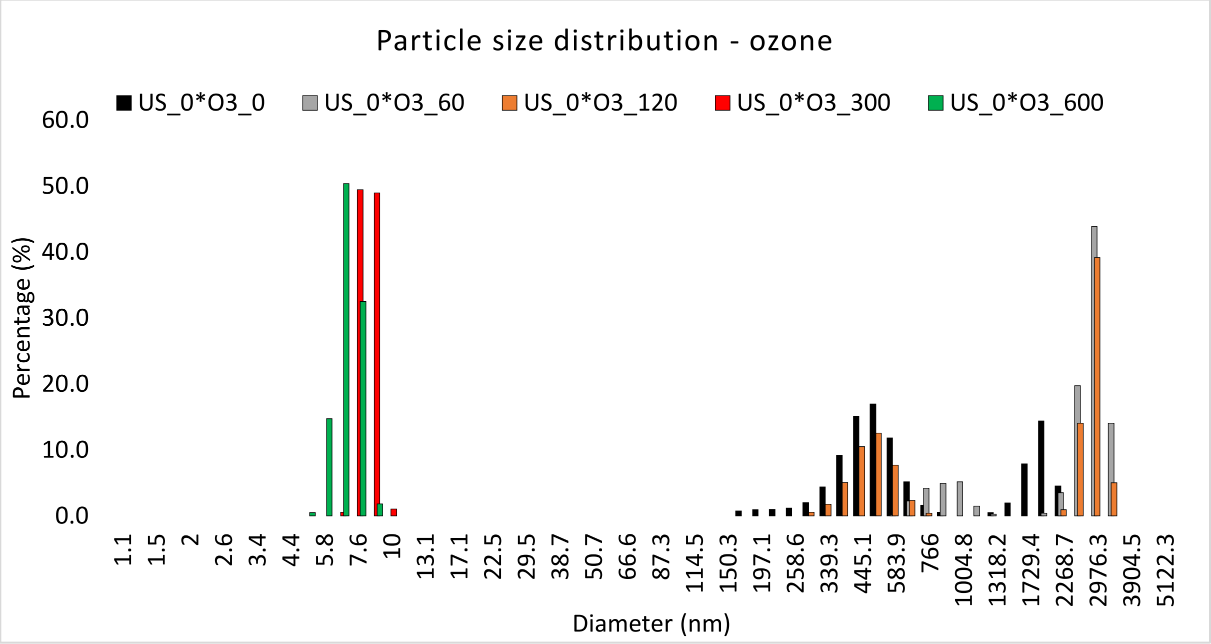

Figure 4-e: Ozone particle size distribution

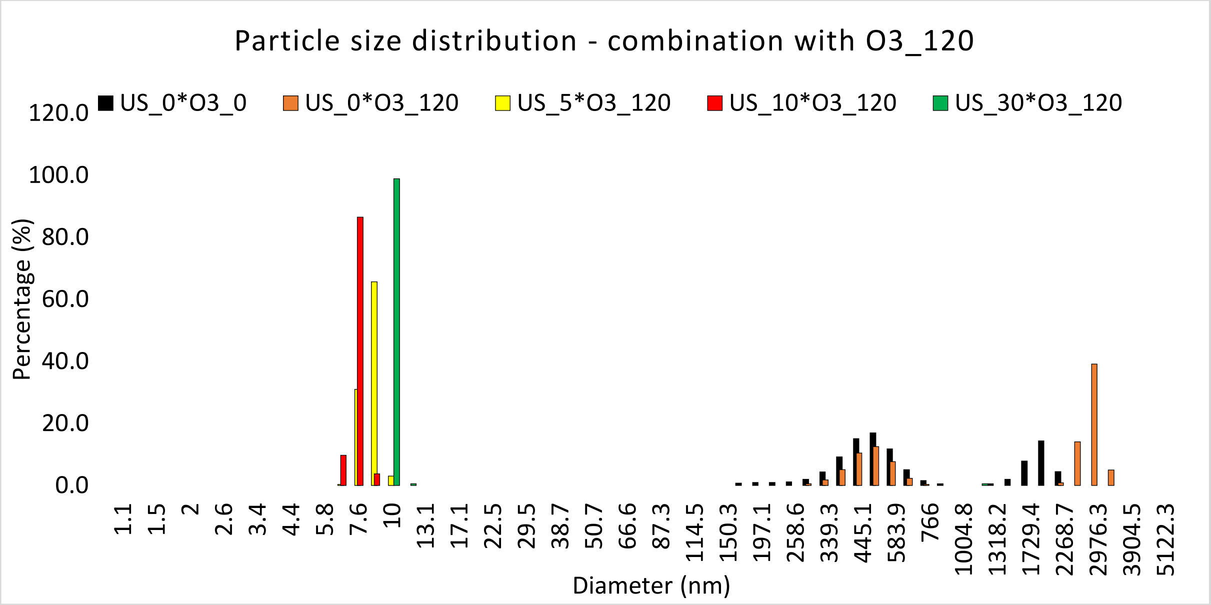

Figure 4-f: Combination with O_3_120 particle size distribution

As a conclusion, the particle size of the WW shifted from hundred-thousand nm to units after the application of 300 and 600 s of ozone (Fig 4-e) or combining at least 5 s of US pretreatment with the 120 s ozonation (Fig 4-f). The diameter's distribution is changing in a limited way in the US only treatment (Fig. 4-d), within the 100 to 3000 nm interval. However, the sole US treatment was not able to reduce markedly the particle size, even after 30 s.

4.1.3 Effects on organic matter

Different methods were employed to evaluate the organic matter content. The legislation requires a mandatory analysis on BOD_5 (in this case substituted by TOC) and COD. A relationship between BOD_5 and TOC was obtained appositively for urban WWTP influents: $BOD_5 = 1{,}68*TOC + 23{,}7$ [97]. The COD assessment was performed in two steps: first the total COD (tCOD) evaluation with the unaltered sample and secondarily the soluble COD (sCOD), after filtering the sample through a 0,45 µm filter.

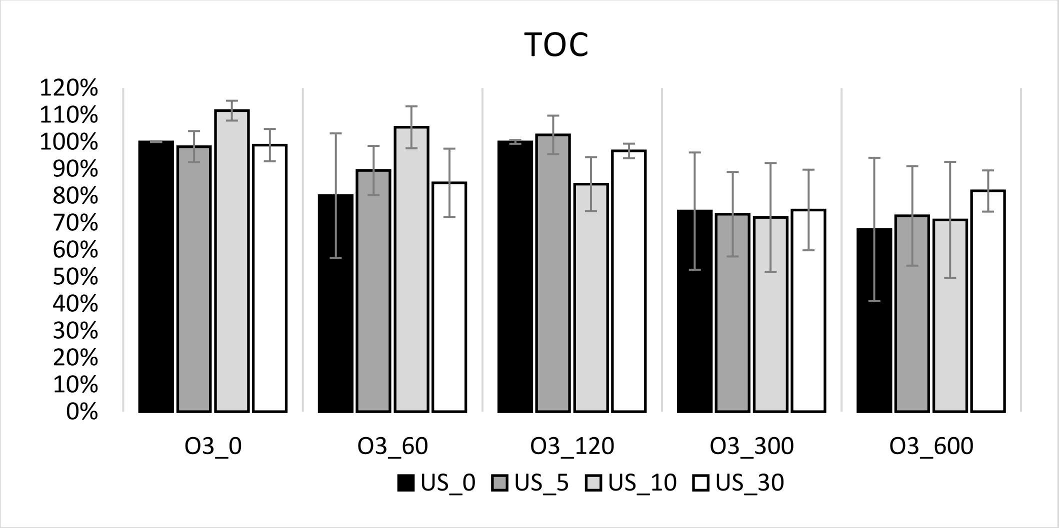

Figure 4-g: TOC removal in sonozone tests

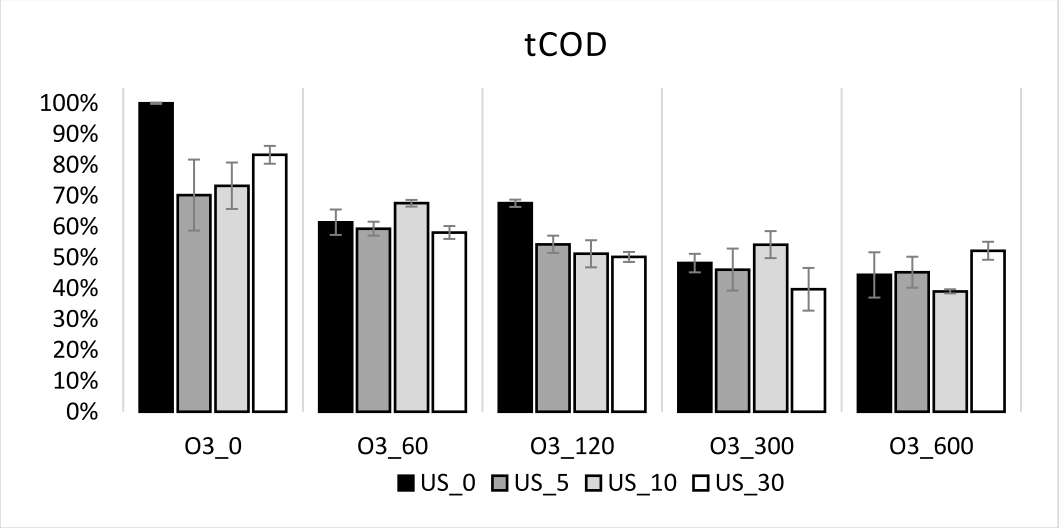

Figure 4-h: Total COD removal in sonozone tests

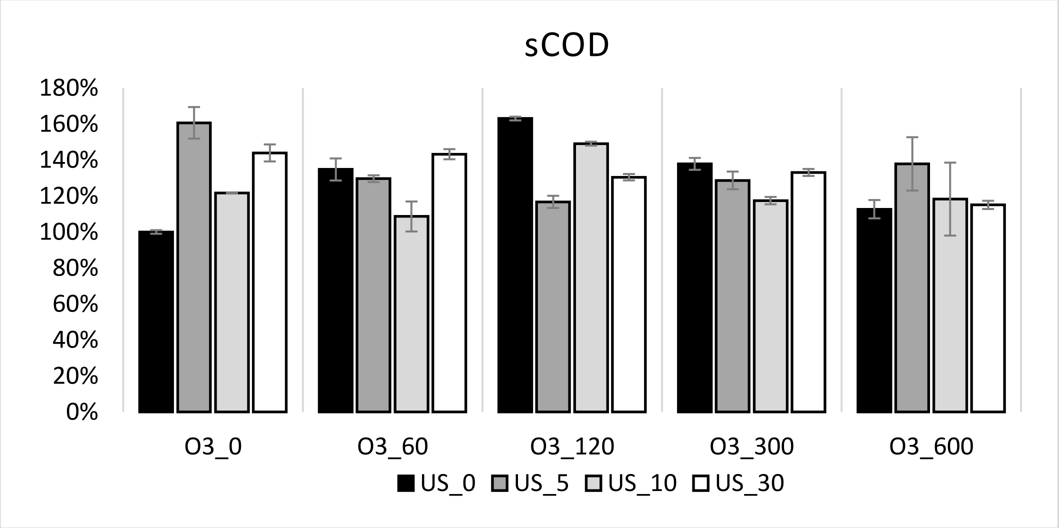

Figure 4-i: Soluble COD removal in sonozone tests

The only US pretreatment led to an initial general enhancement of the TOC concentration, not resulting in a more effective combined action toward the abatement during the following ozonation (Fig. 4-g). However, ozone was able to reduce the TOC concentration up to the 30% with 600 s. Instead of TOC, the tCOD reduction (Fig. 4-h) was more marked, linearly reaching the maximum of 60% of reduction (US_10*O_3_600).

After the application of the US treatment alone, a measurable sCOD increment was observed, even at short sonication times (Fig. 4-i). This outcome could be explained with the physicochemical modification of the mixture during the sonication process that augmented the fraction of soluble organic material. Anyway, differently from the tCOD trend, it is possible to see an augment in the sCOD content also after the ozone application, leading to a non-significant abatement.

Remarkably, UV_{254} is a standardized and adopted spectrophotometric method to assess the organic matter content [98]. The UV_{254} decreasing trend was similar to the tCOD (Fig. 4-j). It is possible to conclude that the combination of US and O_3 may result in an additional 10-20% abatement of the organic matter content inside the WW sample, compared to the ozonation alone. Higher removal percentages were found with the shortest US pretreatment duration. The application of the four O_3_600 s treatments (Fig. 4-j) shown a reverse trend compared to the rest; probably the spectrophotometric signal was distorted by some remained solids.

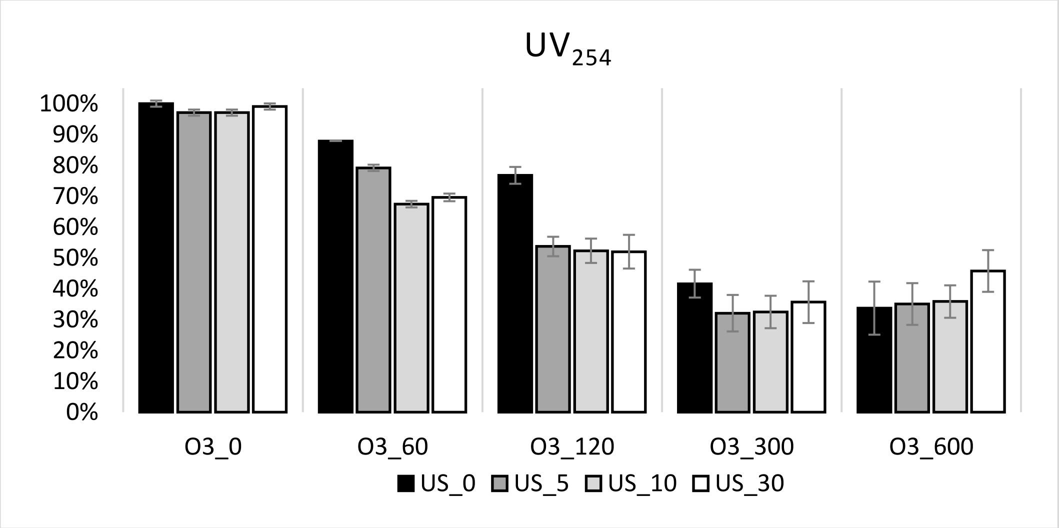

Figure 4-j: UV_{254} removal in sonozone tests

4.1.4 Effects on nutrients

The final purpose of this study is the reuse the nutrients contained inside the wastewater: ozone properties are well-known not to reduce the nitrogen-phosphate content and this is the reason why this process was chosen [95]. The trends of the total measurable N and P are shown in Fig. 4-k and

4-m. The TN analysis confirmed that the sonozone was able to maintain almost the whole content as the untreated sample. A slight augment appeared during the US_10 treatment alone, due to the free compounds released from the surfaces during the cavitation effect, consistent with previous literature studies [99]. In addition, the soluble nitrogen ammonia content was depicted in Fig. 4-l, presenting the same trend as the TN. The overall nitrogen reduction could be established as lower than the 10%. TP was similarly preserved along the process, single and combined. However, about 25% of phosphate was removed by the processes, compared to the 10% removal of TN. P is a meaningful and a scarce nutrient, mandatory for a plant growth. Recovering the 75% of the P originally present in the raw WW means to strengthen the possibilities of RWW reuse for agriculture.

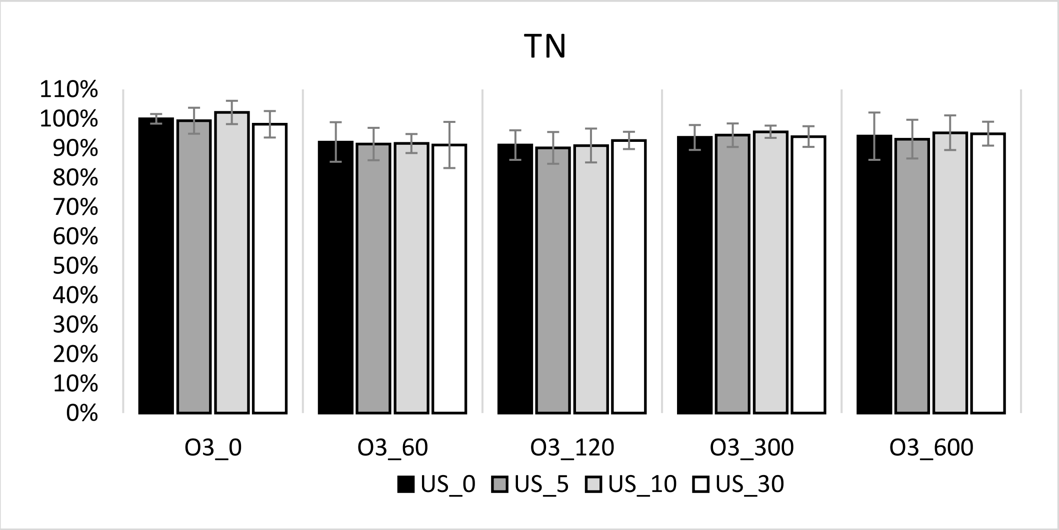

Figure 4-k: TN removal in sonozone tests

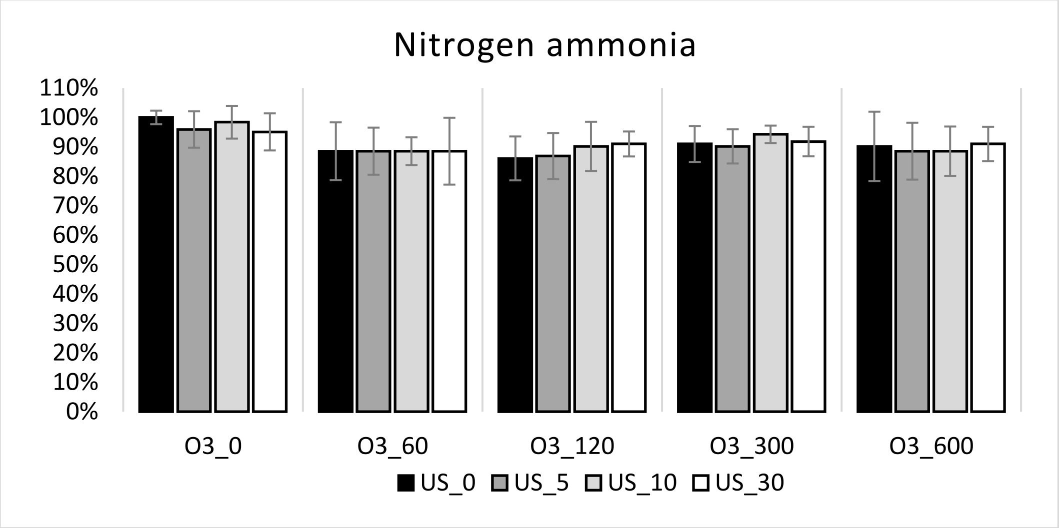

Figure 4-l: Nitrogen ammonia removal in sonozone tests

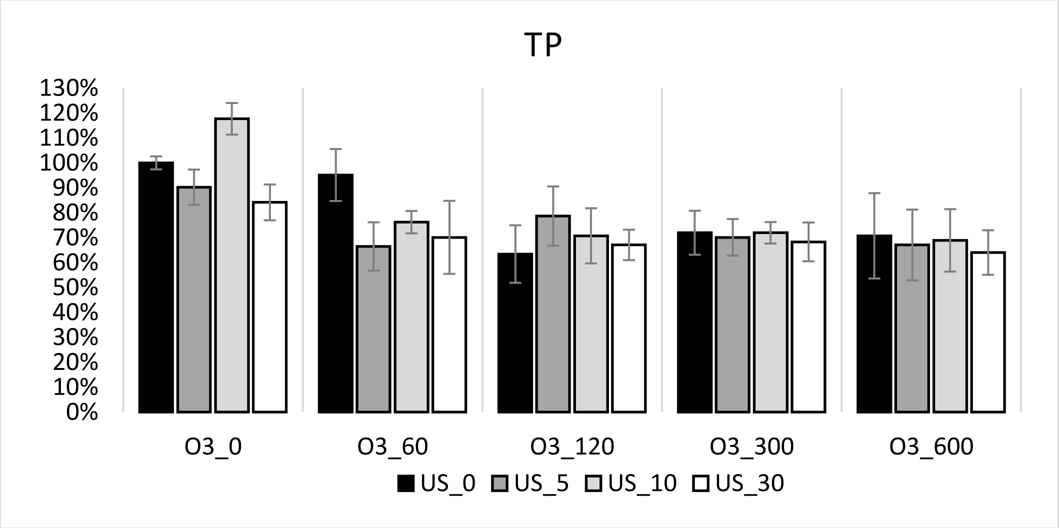

Figure 4-m: TP removal in sonozone tests

Remarkably, for an adequate plant growth also the potassium, calcium and manganese are relevant. The dissolved nutrient concentrations were measured and, comparing the results of the untreated WW with the longest treatment performed during this study (US_30*O_3_600) it was possible to clearly validate the ozone capacity of maintain these nutrients. The results shown an initial concentration of potassium equal to 16,93 mg/L, calcium of 53,38 mg/L and magnesium corresponding of 20,69 mg/L. After the treatments their concentrations were, respectively, 16,31 mg/L, 52,12 mg/L and 20,11 mg/L, verifying the ozone behavior to keep the nutrients.

4.1.5 Effects on microorganisms

The importance of a RWW reuse is not to be harmful for the environment and for the population. So, a complete removal of the pathogens below the regulated thresholds is mandatory. Ozone is a well-known disinfectant, adopted for WW treatment. The US alone shown a negligible effect, no abatement occurred at the above-mentioned low frequency conditions. The application of the combined sonozone treatment shown an aggressive action, thanks to the US pretreatment. Ozone by itself led to a pathogen abatement of one logarithmic unit (Log) after 60 s. The employment of a 30 s of US (US_30*O_3_60) resulted in more than 3 log. The adoption of 120 s or more of ozone treatment, independently of the US action, led to a complete abatement (> 4 log). This complete abatement has to be intended as a non-capacity of counting the microbial colonies on the plates with the maximal dilution performed. It is possible to conclude that the ozone treatment of 120 s or more is able to remove the pathogens, leading to a concentration surely below the 100 CFU/100 mL. The same behavior was outlined in Fig. 4-n. E*nterococcus* spp. highlighted a similar removal trend as the *E. Coli*. Starting from a 6-log concentration, 60 s of ozone was enough to remove from one (only ozone) to 3 (coupled with US_30) logs (Fig. 4-o).

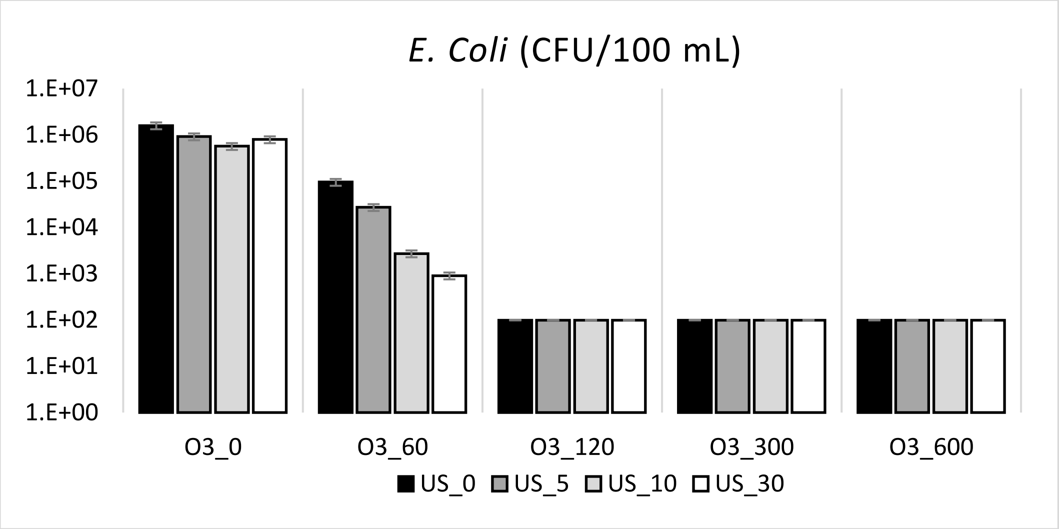

Figure 4-n: E. Coli logarithmic reduction removal in sonozone tests

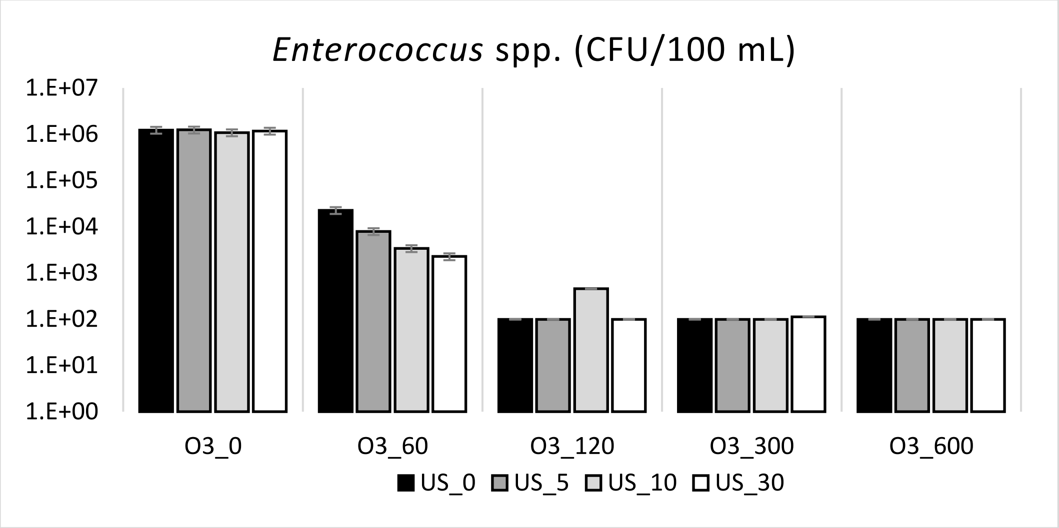

Figure 4-o: Enterococcus spp. logarithmic reduction removal in sonozone tests

Furthermore, salmonella was absent even in the untreated WW assessment. Being a primary importance aspect, the pathogen abatement will be assessed through an in-depth evaluation in the following sections.

4.1.6 Effects on other hazardous pollutants

Formaldehyde was monitored as well, being a well-known ozonation by-product [100]. This compound was present in low concentrations in the untreated sample. An initial abatement was depicted with the only sonication action. In addition, ozone application shown the ability to completely remove the formaldehyde after 120 s of combined application (US_30*O_3_120) or, without pre-treatment, from 300 s on (Fig. 4-p).

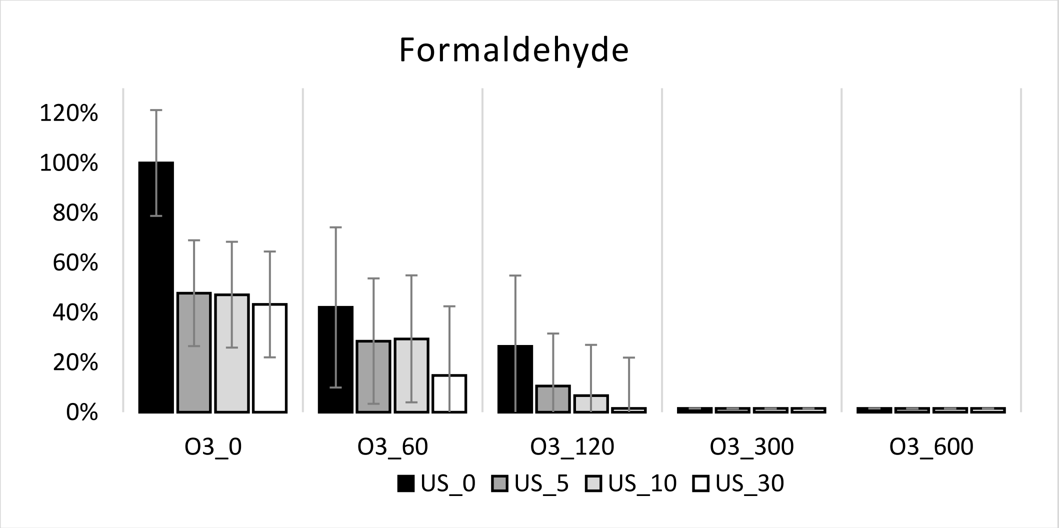

Figure 4-p: Formaldehyde removal in sonozone tests

Furthermore, total surfactants were assessed. Surfactants are defined as chemical compounds capable of reducing the surface tension of liquids or interfaces of liquids, endowed by its hydrophobic tail and hydrophilic head [101]. They could be divided into three main groups: Anionic (Alkylsulphates, alkylsulphonates, alkylarenesulphonates), Cationic (Alkyltrimethylammonium salts, alkylpyridinium salts) and Non-Ionic (Alkylpolyoxyethylane glycol ethers, alkyphenol ethers).

Anionic surfactants are largely diffused in household uses and industrial applications. Their presence is increasing everyday inside the urban WW flow due to their uncontrolled utilization for the cleaning and washing practices [102]. So, being the main components traceable inside the WW PEff, the total quantity of surfactants will be roughly considered equal to the anionic part. Their treatment removal percentages are reported in Fig. 4-q. The employment of the ozone is clearly a strong surfactant reducer, already at O_3_60s. It is possible to define an augment in the percentage of surfactants inside the WW sample after the single applications of US, marking a 40% increment during the only US_30 pretreatment. This result may be related to the cavitation effect that, breaking the large-surface solids inside the sample, releases more free surfactants. Further assessments have to be performed towards the low frequency US application for the anionic surfactant removal. Moreover, the treatment defines a slightly higher abatement for the 60 and 120 s combined application.

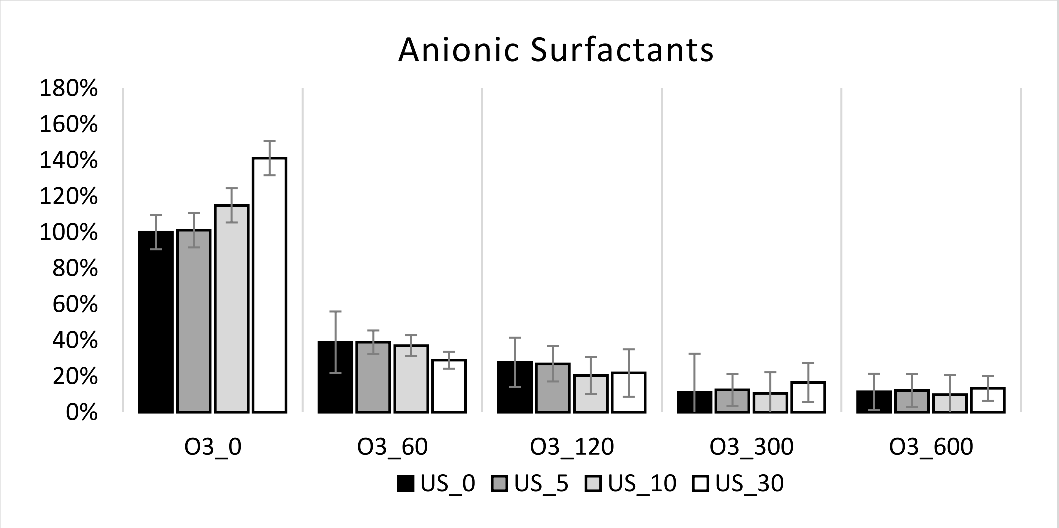

Figure 4-q: Anionic surfactants removal in sonozone tests

4.1.7 Heavy metals and micropollutants assessment

In this paragraph, brief considerations regarding the HMs are given. First of all, their assessment was performed through an ICP instrument. Table 4-2 reports the ministerial directive 185/2003 thresholds (EU legislation still not requires their assessment for the reuse on soils) compared to the untreated influent values. The concentrations from the investigated PEff are all abundantly lower than the limits, confirming its reusability.

Table 4-2: HMs comparison between 185/2003 and actual PEff concentrations

Micropollutant	185/2003 reuse threshold	PEff measured value
Aluminum (mg/L)	1	0,014
Boron (mg/L)	1	0,055
Cadmium (mg/L)	0,005	< 0,001
Cobalt (mg/L)	0,05	0,02
Chromium (mg/L)	0,1	< 0,01
Copper (mg/L)	1	0,003
Iron (mg/L)	2	0,075
Manganese (mg/L)	0,2	0,052
Nickel (mg/L)	0,2	0,003
Lead (mg/L)	0,1	< 0,01
Zinc (mg/L)	0,5	< 0,01

Even the strongest treatment performed (US_30*O_3_600) shown no capacity of mineralization towards the analyzed HMs, at the current ozone concentrations.

4.1.8 Energy assessment

Concerning the economic aspects, even traditional treatment schemes imply high energy consumption: aeration is one of the main cost items, with up to 71% of total WWTP electricity consumption (0,5-2 kWh/m^3, depending on treatment technology) [103]. The recovery of the PEff, treated through the sonozone process, could save this amount of employed energy. However, US and O_3, being well-known energy-consumer processes, have to be assessed on field based on their removal capacity. Table 4-3 reports a basic economic and consumption assessment towards the hybrid employment of US and O_3. The analysis was performed specifically on the unit of volume (m^3) and considering no exceeding volumes. The treatment was supposed as continuously running.

Table 4-3: Basic energetic assessment

Treatment	Treated volume (L)	Specific energy consumption (kWh/m^3)	Specific electric cost (€/m^3)
US_0*O_3_60	0,80	2,81	0,66
US_0*O_3_120	0,80	5,63	1,32
US_0*O_3_300	0,80	14,06	3,29
US_0*O_3_600	0,80	28,13	6,58
US_5*O_3_60	0,80	3,33	0,78
US_5*O_3_120	0,80	6,14	1,44
US_5*O_3_300	0,80	14,58	3,41
US_5*O_3_600	0,80	28,64	6,70
US_10*O_3_60	0,80	3,84	0,90
US_10*O_3_120	0,80	6,65	1,56
US_10*O_3_300	0,80	15,09	3,53
US_10*O_3_600	0,80	29,15	6,82
US_30*O_3_60	0,80	5,90	1,38
US_30*O_3_120	0,80	8,71	2,04
US_30*O_3_300	0,80	17,15	4,01
US_30*O_3_600	0,80	31,21	7,30

Despite being huge electric-consumers, the US and O_3 processes may be optimized while designing a full-scale application [104].

4.1.9 Literature comparison

The single employment of ultrasounds and ozone for WW treatment was reported several times in literature. However, only a few studies assessed the combined effect of the hybrid US and O_3 processes. These studies presented similarities compared to the execution of the current work, even considering different experimental conditions.

Rossi et al. defined the most similar laboratory conditions to the current work [52]. The WW characteristics and the outputs showed corresponding behaviors, maintaining comparable trends for the pollutant reduction and the nutrient preservation.

Sathishkumar et al. summarized several previous studies regarding the hybrid application of ozone and other AOPs, including ultrasounds [105]. However, longer ozonation times and higher ultrasonic frequencies were employed, thus the comparison with the current work is challenging. Moreover, the combined US and O_3 process was performed simultaneously inside a single reactor, differently from the sequential treatment supposed during the current study.

Mahamuni and Adewuyi mainly focused on the cost estimation of the different AOP treatments [106]. US and O_3 combination resulted to be one of the most convenient for the organic matter oxidation, above all the AOPs analyzed, consistent to the results obtained in the current work.

Furthermore, significant enhancements in the contaminant removal rates were obtained during the application of the hybrid process compared to the ozonation alone [107].

The robust literature analysis, describing the single treatments, was adopted as a reference during the initial part of the work, and allowed to design the laboratory experiments with the aim to increase the removal efficiency and optime costs. The novelty of the current study was the transition from the concept of a purely contaminant abatement to the adoption of these AOPs for a combined reduction of organic pollutants and maintenance of the nutrients contained inside the WW, for an agricultural reuse perspective.

4.2 Sonozone: microbial kinetic modelling and cost-effectiveness

The present section aims to investigate the microbial inactivation potential of US and ozonation alone, to recover the WW from a microbiological point of view. To this aim, sterile urban WW was spiked with four microbial species widely present in WW, namely *Pseudomonas* spp*., E. coli*, *Enterococcus* spp. and *S.* Enteritidis. The concentrations of the chemical compounds of the WW used in this study are similar to typical urban treatment plant inflows, as reported in other studies [19], [108]. The US frequency and the ozone concentration were kept consistent with the laboratory-scale experience.

4.2.1 Effect of US on microbial viability

During the experimental campaign, spiked WW was treated for times ranging from 5 to 600 s (at a controlled temperature), and in the case of the longer treatment only a slight decrease in microbial viability was observed, which was not statistically significant. Sonication for 600 s resulted in *Pseudomonas, Enterococcus, E. coli* and *S.* Enteritidis removal of 56%, 38%, 51% and 60%, respectively. Our results agree with previously reported data, that *E. coli* showed a log reduction lower than one even for longer (60 min) and more consuming treatments (100 W) [91]. Similar reductions were reported for other Gram-positive and Gram-negative bacteria [92]. Instead, up to 7 log reductions in the total viable count of bacteria and yeasts could be achieved using high-frequency (850 kHz) US or when US treatment was applied without temperature control, allowing a temperature of about 90 °C to be reached at the end of the treatment [109]–[111].

4.2.2 Effects of ozone treatment

Spiked WWs were submitted to ozonation treatments, with a mean gaseous concentration of 1,46 mg/L and treatment times between zero and 900 s. The inactivation kinetics were assessed through the implementation of the experimental outvalues into the GInaFiT software [75]. For all microbial targets, the viable count decreased by increasing the treatment time. A convex inactivation curve followed by a tail in ozonated WWs suggests that at the very beginning of the treatment all target cells possess the same resistance, and a sub-resistant population might exist indicated by the presence of a tail. For all microbial targets, the main inactivation occurred in the first minutes of treatment. As an example, the *Pseudomonas* spp. inactivation in spiked WW by the ozonation treatment was reported Fig. 4-r.

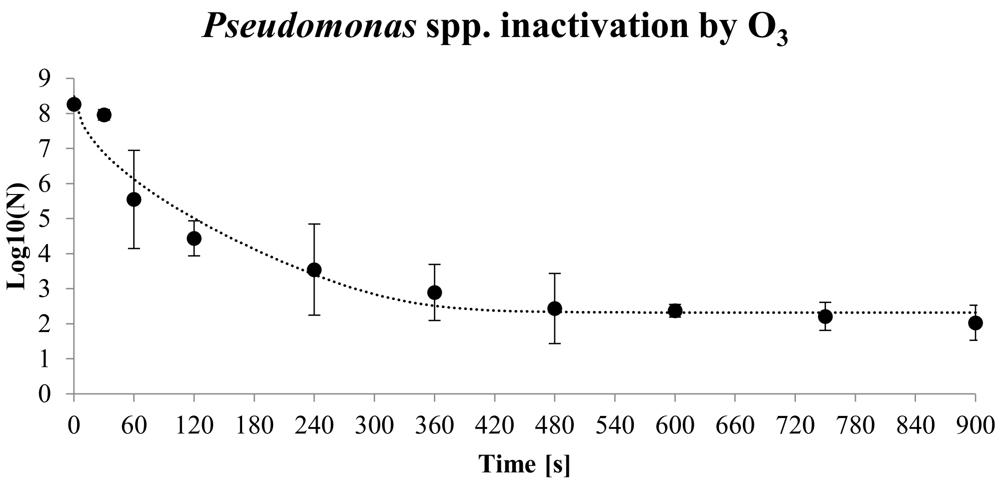

Figure 4-r: Pseudomonas spp. inactivation (Log CFU/mL) in spiked WW by ozonation treatments; dotted line represents data fitted by the Albert and Mafart (2003) model.

S. Enteritidis was the most sensitive organism, whose 90% of cells resulted no more viable after just about 6 s of ozonation. *Pseudomonas* spp. and *Enterococcus* spp. proved to be the most tough microbial targets to inactivate through ozonation. Enterococci are opportunistic pathogens of enteric origin, and they are very resistant to environmental stresses, since they could modulate gene expression, which involves a metabolic reprogramming and a cellular state of increased resistance to many stressful conditions. Compared to other fecal microorganisms, Enterococci survive longer in aquatic environments, even after O_3 treatment [112], [113]. The data indicate that the ozonation treatments carried out in this study were found to be very effective in reducing the viability of microbial targets frequently present in WW, that are considered in most cases indicators of contamination of fecal origin. This suggests the great potential of ozonation for the microbiological recovery of WW.

4.2.3 Effects of sonozone treatment

In the next phase of the study, ultrasound and ozone were sequentially applied on spiked WWs. Four US treatments were applied (i.e., US_30, US_90, US_300 and US_600 s). As for ozone, two treatments were chosen for each microbial genus based on the time estimated to get the first decimal reduction and 4 log reductions in the initial viable count. Thus, O_3_60 and O_3_120 for *Pseudomonas* spp., O_3_30 and O_3_60 for *Enterococcus* spp., O_3_15 and O_3_30 for *E. coli*, and O_3_30 and O_3_45 for *S*. Enteritidis were selected, respectively.

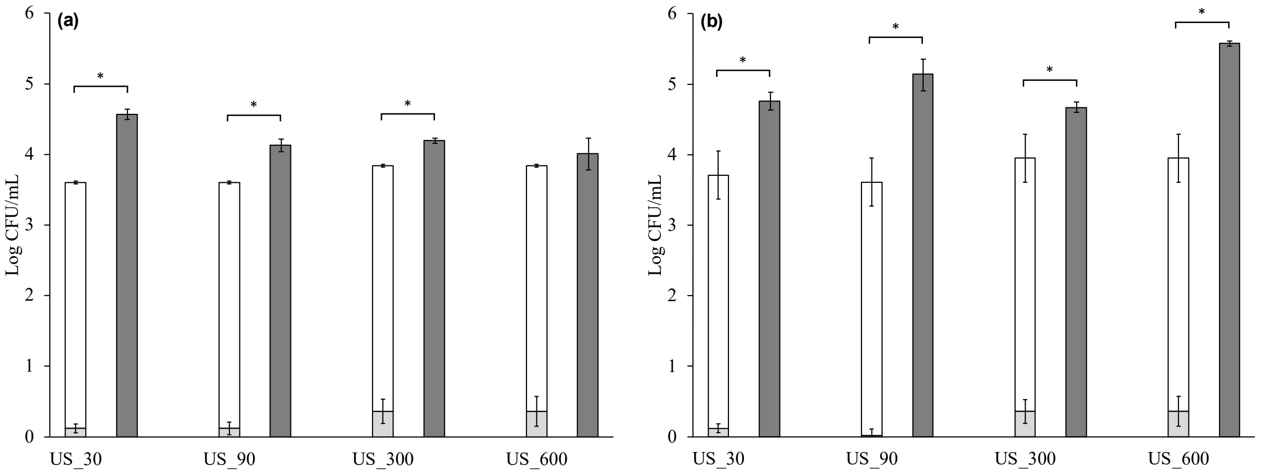

Figure 4-s: Log reduction of Pseudomonas spp. viability following US treatments (light grey bars), O_3 treatments (white bars) for 60 s (a) and 120 s (b), and sonozone treatments (dark grey bars). Asterisks mean a statistical difference between US and ozone applied alone and in combination.

Fig. 4-s shows the reductions in viability resulting from treatments with US and O_3 applied individually and in succession on *Pseudomonas* spp. Most of the combined treatments allowed to significantly reduce the microbial viability compared to the sum of the single treatments, which suggested a synergistic effect of them. Except for the US_600 followed by O_3_60 treatment, the increased loss of viability in the combined treatments varied between 0,36 and 1,63 Log CFU/mL. The most effective treatment was US_600 followed by O_3_120, which killed 5,58 Log CFU/mL of *Pseudomonas* spp. Overall, despite the reduced loss of viability following the US treatment alone, O_3 treatments applied just after US proved to be very effective against *Pseudomonas*. Vice versa, a combined treatment with US and O_3 reduced the viability by 99%. US, although ineffective even for a long time, can transform a portion of the microbial population into a VBNC (Viable But Not Culturable) status, which means an intermediate status between live and dead cells [91]. In the case of Gram-negative bacteria, US is firstly effective on the outer membrane, subsequently, the treatment becomes multi-target, involving the cell wall, the cytoplasmic membrane, and the internal structure [114]. Results obtained in this study represent an important finding as *Pseudomonas* is one of the main contaminants of WW [115].

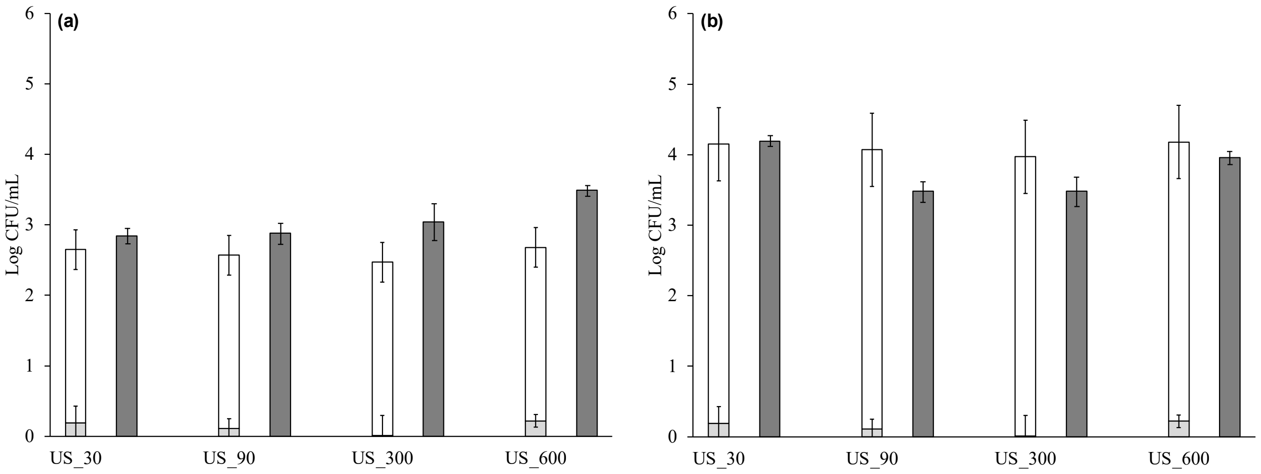

Figure 4-t: Log reduction of Enterococcus spp. viability following US treatments (light grey bars), O_3 treatments (white bars) for 30 s (a) and 60 s (b), and sonozone treatments (dark grey bars).

As for *Enterococcus* spp., the only treatments that proved capable of increasing the loss of viability respect to pure O_3 treatments were with US for 300 s and 600 s followed by O_3 for 30 s, in which increased loss of viability was 0,57 and 0,81 Log CFU/mL, respectively (Fig. 4-t). Unexpectedly, the combined treatments with O_3 for 60 s did not boost the killing effect respect to O_3 only. It could indicate that the microbial response to US may involve not only structural damage but also differential gene expression. It has been evidenced that, when bacteria are subjected to an US treatment below the bacterial tolerance threshold, mechano-transduction may occur, which activates the bacterial stress response [116]. The application of low-frequency ultrasound treatments, such as in this study, can induce a downregulation in the genes involved in the citric acid cycle and respiratory chain, as well as in ABC transporters, which results in the decrease of ATP production and reduced membrane permeability [114]. However, after such treatment bacteria activate the DNA and protein repair systems, which might explain the relative tolerance of *Enterococcus* to many combined treatments applied in this study [117].

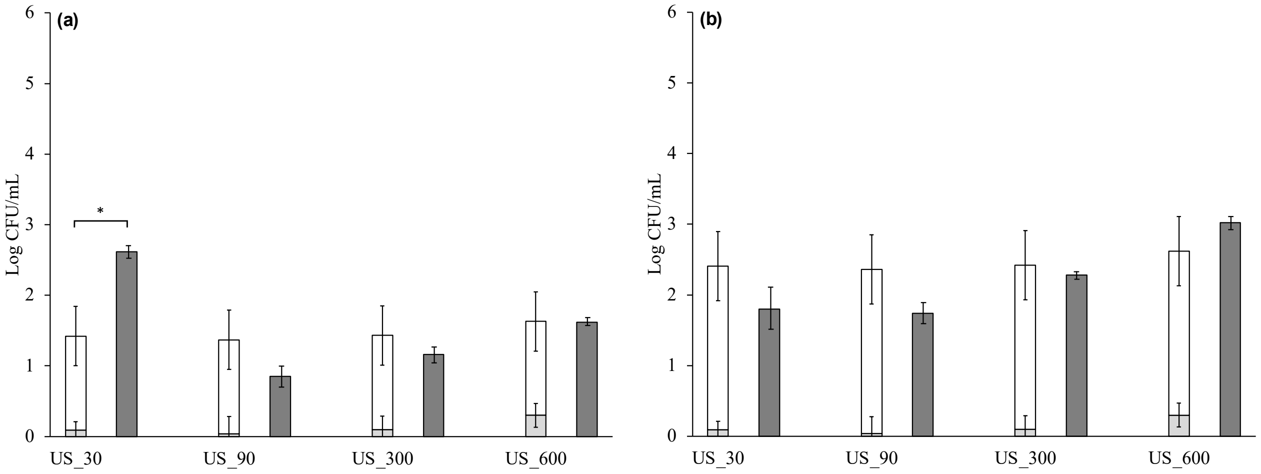

Figure 4-u: Log reduction of E. coli viability following US treatments (light grey bars), O_3 treatments (white bars) for 15 s (a) and 30 s (b), and sonozone treatments (dark grey bars). Asterisks mean a statistical difference between US and ozone applied alone and in combination.

No effects were observed on *E. coli* for combined treatment when O_3 was applied for 30 s, and a significative viability reduction was detected only in the case of the combined treatment with the shortest US and O_3 times, i.e., 30 s and 15 s, respectively (Fig. 4-u).

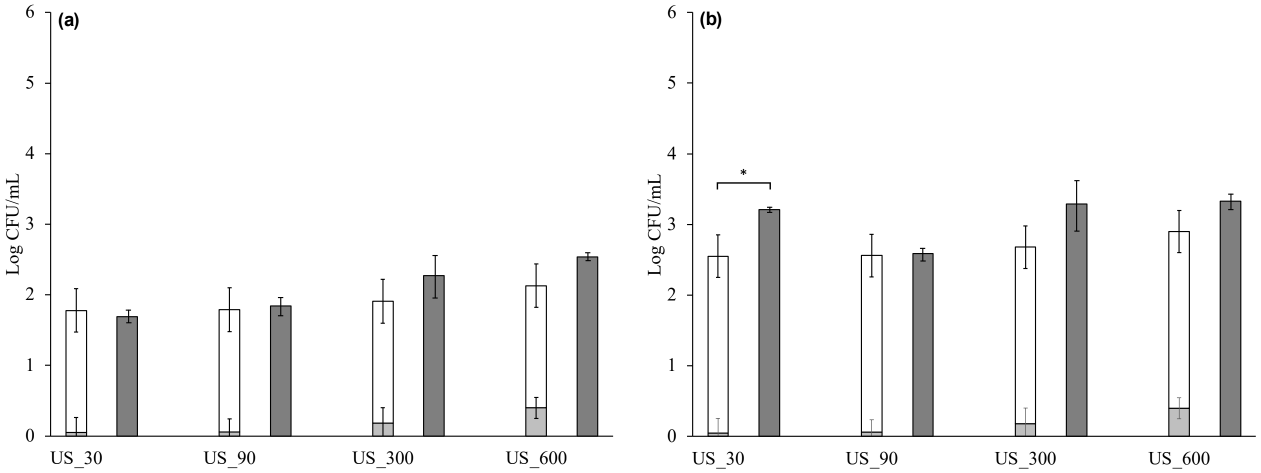

Figure 4-v: Log reduction of S. Enteritidis viability following US treatments (light grey bars), O_3 treatments (white bars) for 30 s (a) and 45 s (b), and sonozone treatments (dark grey bars). Asterisks mean a statistical difference between US and ozone applied alone and in combination.

As for *S*. Enteritidis, its viability was significantly reduced by US_30 followed by O_3_45 respect to US and O_3 applied alone (Fig. 4-v). The antimicrobial effect of combined ozone plus ultrasound treatments on *Salmonella* were only reported to improve safety of plant foods, such as cabbage, spinach, and lettuce [118]–[120], whereas to the best of our knowledge no data are present in the literature on the application of such treatments in WW. On the other hand, the combination of ozone and ultrasound treatments was previously proven to be effective against *E. coli* in saline water (NaCl 0,9% w/v). US boosted the effect of O_3 through the declumping of bacterial clusters and the dispersion of cells that are more sensitive to the ozone. Moreover, cavitation occurs, affecting the cell membrane, as well as free radicals' formation increases, thus enhancing the overall antimicrobial effect. It was also observed that, after a combined ultrasound and ozone treatment, *E. coli* lost the cell wall, and possibly due to a synergistic effect of US and O_3 [91].

4.2.4 Cost-effective analysis

In this section, an economic evaluation is outlined and applied to the sonozone experiments. The achievement of a reliable technology, suitable for any treatment application, have to possess two main characteristics: technical feasibility and economic advantages. The consumption and cost assessments are essential for designing and optimizing a process [121]. The laboratory tests gave remarkable results in a view to a pathogenic reduction. However, higher removal rates do not regularly correspond to a cost-effective energy consumption. Towards an optimization of the process and a bigger up-scale applicability, the parameters $E_{s\%}$ and ΔM are measured. In WWTPs, the processes scaling up demonstrated to be suitable towards a reduction of the overall energy consumptions [122]. The proposed simplified approach could validate the application of the combined US and O_3 treatment within a disinfection process line.

When the $E_{s\%} > 0$ (*Eq. 7*) the combined sonozone process may be considered as economically convenient compared to the separate processes.

Table 4-4: Microbial cost-effective analysis

Pathogen	Treatment combination	$E_{s\%}$ (%)	ΔM (€/m3)
***Pseudomonas* spp.**	US_30*O_3_60	67%	12,0
	US_90*O_3_60	55%	9,0
	US_300*O_3_60	43%	9,5
	US_600*O_3_60	30%	8,3
	US_30*O_3_120	42%	8,1
	US_90*O_3_120	46%	11,0
	US_300*O_3_120	30%	7,5
	US_600*O_3_120	38%	15,1
E. coli	US_30*O_3_15	43%	1,5
	US_90*O_3_15	-14%	-0,4
	US_300*O_3_15	-1%	-0,1
	US_600*O_3_15	3%	0,4
	US_30*O_3_30	-26%	-0,7
	US_90*O_3_30	-19%	-0,8
	US_300*O_3_30	-2%	-0,2
	US_600*O_3_30	4%	0,7
***Enterococcus* spp.**	US_30*O_3_30	32%	1,6
	US_90*O_3_30	25%	1,6
	US_300*O_3_30	15%	1,8
	US_600*O_3_30	11%	2,1
	US_30*O_3_60	1%	0,1
	US_90*O_3_60	-7%	-0,5
	US_300*O_3_60	-4%	-0,5
	US_600*O_3_60	-1%	-0,1
***S.* Enteritidis**	US_30*O_3_30	-35%	-0,9
	US_90*O_3_30	-11%	-0,5
	US_300*O_3_30	7%	0,8
	US_600*O_3_30	9%	1,8
	US_30*O_3_45	43%	3,5
	US_90*O_3_45	10%	0,7
	US_300*O_3_45	26%	3,9
	US_600*O_3_45	19%	4,2

Several combinations resulted to be advantageous (Table 4-4). The trend showed convenient energy percentages, particularly while applying a short ultrasonic pre-treatment right before the ozone process. Notably, *Pseudomonas* spp. gave significant energy usage reductions, up to the 67% for the US_30*O_3_60 combination. Anyway, the application of the 30 s ultrasound treatment demonstrated to be able to reduce the energy usage more efficiently than the longer US pre-treatments, among all the analyzed pathogens. In favor of a disinfection of several meter cubes per day, the same considerations were given towards the cost reduction. Selecting the most efficient sonozone combinations it is possible to obtain a theorical reduction of spent money ΔM equal to 12,0 €/m^3 (*Pseudomonas* spp. US_30*O_3_60) and 15,1 €/m^3 (*Pseudomonas* spp. US_600*O_3_120). Despite being the most resistant pathogen, *Pseudomonas* spp. shown the most advantageous percentages. Longer ozone applications (60 s and 120 s) could have given the possibility for the oxidant to be more efficient. Advantageous savings percentages were again demonstrated while applying shorter US pretreatments (30 s). *E. Coli* shown unstable results and, probably due to the short ozonation times, often not advantageous. *Enterococcus* spp. (30 s treatment) and *S*. enteritidis (45 s treatment) gave suitable results, in a view of a WW microbial reusability. The negative values reported in Table 4-4 have to be considered as not favorable energetically or economically (combined treatment power usage higher than the single one).

4.3 Sonozone: ecotoxicological in-silico evaluations

The chemical compounds considered for the in-silico evaluation were indicated from the GC-MS and the HPLC-HRMS analysis. The results, shown in Table 4-5 and 4-8, will be presented as percentage reduction, comparing the concentration traceable in the NT sample to the after-treatment value (< 100% reduction; > 100% augment; 100% not efficient treatment). However, particularly during the GC-MS analysis, new volatile compounds may be created after the oxidation of the initial organic compounds. The GC-MS outputs, composed by aldehydes, ketones and alcohols, will be assembled into two main categories (Aromatic and Aliphatic). Differently, the several HPLC-HRMS analyzed compounds were gathered into more categories: supplements, pharmaceuticals, nylon, health care products, cleaning products, insect repellents, steroids, health care products, perfumes & PVC, drugs and dyes. For all the categories, the software simulated the models of “fish acute” and “fish chronic”, “Daphnia Magna acute” and “Daphnia Magna chronic”, “algae acute” and “algae chronic”, “persistence in soils” and “persistence in water” and “water solubility”. For the explanation of the following tables, it is possible to divide the toxicity levels in four ranges of concentrations: > 100 mg/L – non-toxic (nT); between 10 and 100 mg/L – low toxicity (L-T); between 1 and 10 mg/L – medium toxicity (M-T) and < 1 mg/L – high toxicity (H-T). In the same way, persistence is categorized in three ranges: < 120 days – non-persistent (nP); between 120 and 180 days – persistent (P) and > 180 days – very persistent (vP).

Starting from the aromatic and aliphatic compounds, from Table 4-5 is possible to conclude that all the initial compounds in the NT sample were oxidated after 120 s of ozone, single or combined. US pretreatment was slightly able to enhance their removal. Moreover, 2-Nonenal, 2-pentyl-, Heptanal and Nonanal were three aldehydes formed after the application of the O_3_120, confirming that ozone may produce aldehydes as a by-product, while applying the ozonation for more than 60 s. However, US_30*O_3_120 and even more O_3_600 oxidated the totality of them. The most hazardous aromatic and aliphatic compounds resulted to be the 3-Octanol, 3,7-dimethyl (H-T for Daphnia Magna), the Heptanal (H-T for Daphnia Magna) and Phenol, 4-(1,1,3,3-tetramethylbutyl)- (H-T for algae) (Table 4-6). The sonozone treatment demonstrated to be able to remove these dangerous compounds. Above all, Heptanal is an aldehyde formed during the ozone process. The application of long O_3 treatments or a 30 s US pretreatment could remove it from the WW. Inside this selection of aromatic and aliphatic chemicals, no one shown remarkable persistence in soils or water, and their solubility in water was below one mg/L exclusively for the Phenol, 4-(1,1,3,3-tetramethylbutyl)- (Table 4-7).

The removal rate of supplements, pharmaceuticals, nylon components, health care products, cleaning products, insect repellents, steroids, health care products, perfumes & PVC, drugs and dyes was different from the previously described aromatic and aliphatic compounds. Exclusively the longest ozonation time led to a high percentage of abatements, while the US pretreatment was often ineffective against these compounds (Table 4-8). Moreover, the Triethylene glycol, a pharmaceutical compound, resulted to be present in a higher concentration after the US pretreatments, compared to the untreated sample and the hybrid process was not capable of oxidize it. The abatement rates of other compounds seemed to be meaningful for the O_3_600 and only partial for shorter ozone durations. The most toxic detected compound were: Leucine, Isoleucine and N,N Diethyltoluamide (H-T for fish), Diethylphthalate, Laurolactam and Tyrosine (H-T for Daphnia Magna) and the Triethylene glycol (H-T for both Daphnia magna and algae)(Table 4-9). This last pharmaceutical compound is the toughest one to be removed and one of the most toxic presents within the researched ones. The soil persistence revealed no troublesome compounds. However, the assessment identified Diethylphthalate, N,N Diethyltoluamide and Tyrosine as very persistent in water. Furthermore, low solubility concentration is not typical among these compounds (Table 4-10).

Table 4-5: Aromatic and aliphatic compound reduction percentages

Compound	N.T.	US_0* O_3_60	US_0* O_3_120	US_0* O_3_600	US_5* O_3_60	US_5* O_3_120	US_5* O_3_600	US_30* O_3_60	US_30* O_3_120	US_30* O_3_600
1-Hexanol, 2-ethyl-	100%	71%	0%	0%	118%	0%	0%	54%	0%	0%
2-Nonenal, 2-pentyl-	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
3-Octanol, 3,7-dimethyl-	100%	50%	0%	0%	53%	0%	0%	31%	0%	0%
Ethanone, 1-(2,2-dimethylcyclopentyl)-	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Heptanal	0%	0%	100%	0%	0%	62%	0%	0%	0%	0%
Nonanal	0%	0%	100%	0%	0%	89%	0%	42%	25%	0%
Phenol, 2,4-bis(1,1-dimethylethyl)-	100%	23%	0%	0%	34%	0%	0%	27%	0%	0%
Phenol, 4-(1,1,3,3-tetramethylbutyl)-	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table 4-6: Aromatic and aliphatic compound ecotoxicity assessment

				Ecotoxicity								
				Fish			Daphnia Magna			Algae		
Compound	Aliphatic or Aromatic	Group	Formula	Acute [mg/L]	Chronic [mg/L]	Rank	Acute [mg/L]	Chronic [mg/L]	Rank	Acute [mg/L]	Chronic [mg/L]	Rank
1-Hexanol, 2-ethyl-	Aliphatic	Alcohol	$C_8H_{18}O$	31,24	0,29	**L-T**	11,11	1,60	**L-T**	7,33	2,69	**M-T**
2-Nonenal, 2-pentyl-	Aliphatic	Aldehyde	$C_{14}H_{26}O$	60,65	0,39	**L-T**	6,35	0,48	**M-T**	4,12	1,34	**M-T**
3-Octanol, 3,7-dimethyl-	Aliphatic	Alcohol	$C_{10}H_{22}O$	1,49	0,03	**M-T**	0,25	0,19	**H-T**	1,27	0,14	**M-T**
Ethanone, 1-(2,2-dimethylcyclopentyl)-	Aromatic	Keton	$C_9H_{16}O$	67,13	3,98	**L-T**	6,30	1,66	**M-T**	82,31	7,57	**L-T**
Heptanal	Aliphatic	Aldehyde	$C_7H_{14}O$	1,54	0,14	**M-T**	0,20	0,13	**H-T**	1,01	0,27	**M-T**
Nonanal	Aliphatic	Aldehyde	$C_9H_{18}O$	5,35	0,30	**M-T**	7,90	0,20	**M-T**	3,40	0,47	**M-T**
Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic	Alcohol	$C_{15}H_{24}O$	18,36	1,42	**L-T**	17,78	2,68	**L-T**	17,18	3,55	**L-T**
Phenol, 4-(1,1,3,3-tetramethylbutyl)-	Aromatic	Alcohol	$C_{14}H_{22}O$	4,71	0,03	**M-T**	1,80	0,22	**M-T**	0,80	0,06	**H-T**

Table 4-7: Aromatic and aliphatic compounds persistence and solubility

				Environmental controls				Physic-chemical properties
				Persistence in soils		Persistence in water		Water solubility
Compound	**Aliphatic or Aromatic**	**Group**	**Formula**	**Half-life [days]**	**Class**	**Half-life [days]**	**Class**	**Concentration [mg/L]**
1-Hexanol, 2-ethyl-	Aliphatic	Alcohol	$C_8H_{18}O$	2	**nP**	8	**nP**	874,57
2-Nonenal, 2-pentyl-	Aliphatic	Aldehyde	$C_{14}H_{26}O$	2	**nP**	8	**nP**	542,35
3-Octanol, 3,7-dimethyl-	Aliphatic	Alcohol	$C_{10}H_{22}O$	7	**nP**	60	**nP**	29,73
Ethanone, 1-(2,2-dimethylcyclopentyl)-	Aromatic	Keton	$C_9H_{16}O$	7	**nP**	7	**nP**	1531,86
Heptanal	Aliphatic	Aldehyde	$C_7H_{14}O$	5	**nP**	4	**nP**	40,49
Nonanal	Aliphatic	Aldehyde	$C_9H_{18}O$	7	**nP**	16	**nP**	95,36
Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic	Alcohol	$C_{15}H_{24}O$	7	**nP**	5	**nP**	1241,68
Phenol, 4-(1,1,3,3-tetramethylbutyl)-	Aromatic	Alcohol	$C_{14}H_{22}O$	26	**nP**	6	**nP**	0,56

Table 4-8: Pollutant reduction percentages

Compound	**N.T.**	**US_0* O_3_60**	**US_0* O_3_120**	**US_0* O_3_600**	**US_5* O_3_60**	**US_5* O_3_120**	**US_5* O_3_600**	**US_30* O_3_60**	**US_30* O_3_120**	**US_30* O_3_600**
2,4,6-Trimethylbenzenesulfonic acid	100%	86%	70%	2%	87%	79%	5%	85%	78%	5%
4-Decylbenzenesulfonic acid	100%	29%	13%	2%	44%	22%	3%	39%	21%	1%
4-Dodecylbenzenesulfonic acid	100%	21%	14%	4%	31%	16%	4%	30%	17%	3%
4-Undecylbenzenesulfonic acid	100%	25%	13%	2%	41%	19%	3%	38%	20%	2%
Adenosine	100%	49%	22%	1%	112%	31%	1%	150%	74%	1%
Adipamide	100%	100%	73%	62%	85%	70%	61%	76%	69%	56%
Azelaic acid	100%	89%	79%	14%	145%	101%	18%	110%	116%	24%
Cholic acid	100%	54%	29%	0%	57%	40%	0%	54%	37%	0%
Cytidine	100%	60%	4%	0%	94%	13%	0%	92%	34%	0%
Deoxycholic acid	100%	41%	30%	0%	76%	47%	1%	72%	48%	0%
Deoxyuridine	100%	38%	0%	1%	58%	7%	0%	52%	16%	0%
Diethylphthalate	100%	100%	94%	18%	97%	88%	29%	89%	89%	19%
Laurolactam	100%	46%	31%	2%	55%	34%	2%	44%	30%	2%
Laurylbetaine	100%	23%	9%	2%	41%	15%	2%	38%	17%	1%
Leucine + Isoleucine	100%	37%	3%	0%	61%	13%	1%	65%	31%	2%
N,N Diethyltoluamide	100%	81%	65%	5%	81%	69%	6%	75%	68%	5%
Theophylline	100%	33%	31%	0%	30%	39%	0%	34%	40%	0%
Triethylene glycol	100%	128%	129%	139%	136%	138%	160%	118%	138%	140%
Tyrosine	100%	31%	2%	1%	61%	10%	0%	57%	21%	1%
Xantine	100%	46%	2%	0%	91%	14%	0%	115%	25%	0%

Table 4-9: Pollutant ecotoxicity assessment

			Ecotoxicity								
			Fish			Daphnia Magna			Algae		
Compound	Category	Formula	Acute [mg/L]	Chronic [mg/L]	Rank	Acute [mg/L]	Chronic [mg/L]	Rank	Acute [mg/L]	Chronic [mg/L]	Rank
2,4,6-Trimethylbenzenesulfonic acid	Cleaning products	$C_9H_{12}O_3S$	115,91	7,94	nT	90,58	12,41	L-T	41,31	18,00	L-T
4-Decylbenzenesulfonic acid	Cleaning products	$C_{16}H_{26}O_3S$	109,90	2,38	nT	18,09	6,14	L-T	104,80	4,70	nT
4-Dodecylbenzenesulfonic acid	Drugs and dyes	$C_{18}H_{30}O_3S$	61,30	3,48	L-T	102,83	12,70	nT	48,97	5,80	L-T
4-Undecylbenzenesulfonic acid	Cleaning products	$C_{17}H_{28}O_3S$	25,54	0,90	L-T	14,27	6,13	L-T	2,91	3,59	M-T
Adenosine	Pharmaceuticals	$C_{10}H_{13}N_5O_4$	29,56	1,36	L-T	11,12	5,34	L-T	84,63	7,93	L-T
Adipamide	Nylon	$C_6H_{12}N_2$	96,61	2,69	L-T	81,17	8,56	L-T	110,05	11,24	nT
Azelaic acid	Health care products	$C_9H_{16}O_4$	25,08	0,17	L-T	30,83	11,82	L-T	17,05	3,40	L-T
Cholic acid	Steroids	$C_{24}H_{40}O_5$	23,23	0,33	L-T	16,86	0,26	L-T	10,83	0,68	L-T
Cytidine	Pharmaceuticals	$C_9H_{13}N_3O_5$	63,79	0,83	L-T	44,15	7,55	L-T	78,27	7,26	L-T
Deoxycholic acid	Steroids	$C_{24}H_{40}O_4$	15,31	0,16	L-T	25,45	0,64	L-T	26,85	17,35	L-T
Deoxyuridine	Pharmaceuticals	$C_9H_{12}N_2O_5$	58,17	0,35	L-T	49,40	7,18	L-T	52,04	31,89	L-T
Diethylphthalate	Perfumes & PVC	$C_{12}H_{14}O_4$	2,31	0,46	M-T	0,04	7,06	H-T	6,49	0,15	M-T
Laurolactam	Nylon	$C_{12}H_{23}NO$	2,84	0,20	M-T	0,98	7,83	H-T	4,49	0,66	M-T
Laurylbetaine	Health care products	$C_{16}H_{33}NO_2$	14,99	0,34	L-T	2,53	1,38	M-T	2,30	1,48	M-T
Leucine + Isoleucine	Supplements	$C_6H_{13}NO_2$	0,88	0,02	H-T	1,13	0,02	M-T	6,59	0,66	M-T
N,N Diethyltoluamide	Insect repellents	$C_{12}H_{17}NO$	0,92	0,03	H-T	1,05	0,01	M-T	6,19	0,43	M-T
Theophylline	Pharmaceuticals	$C_7H_8N_4O_2$	41,36	0,35	L-T	21,73	13,93	L-T	52,10	15,93	L-T
Triethylene glycol	Pharmaceuticals	$C_6H_{14}O_4$	1,67	0,18	M-T	0,78	2,83	H-T	0,55	0,16	H-T
Tyrosine	Supplements	$C_9H_{11}NO_3$	2,46	0,03	M-T	0,98	0,01	H-T	5,90	0,37	M-T
Xantine	Pharmaceuticals	$C_5H_4N_4O_2$	33797,21	2,62	nT	87,70	2,28	L-T	26,95	9,41	L-T

Table 4-10: Pollutant persistence and solubility

			Environmental controls				Physic-chemical properties
			Persistence in soils		Persistence in water		Water solubility
Compound	**Category**	**Formula**	**Half-life [days]**	**Class**	**Half-life [days]**	**Class**	**Concentration [mg/L]**
2,4,6-Trimethylbenzenesulfonic acid	Cleaning products	$C_9H_{12}O_3S$	2	**nP**	10	**nP**	21419,47
4-Decylbenzenesulfonic acid	Cleaning products	$C_{16}H_{26}O_3S$	13	**nP**	6	**nP**	699,53
4-Dodecylbenzenesulfonic acid	Drugs and dyes	$C_{18}H_{30}O_3S$	2	**nP**	2	**nP**	26642,58
4-Undecylbenzenesulfonic acid	Cleaning products	$C_{17}H_{28}O_3S$	5	**nP**	21	**nP**	477,34
Adenosine	Pharmaceuticals	$C_{10}H_{13}N_5O_4$	2	**nP**	4	**nP**	2391,68
Adipamide	Nylon	$C_6H_{12}N_2$	5	**nP**	6	**nP**	69,03
Azelaic acid	Health care products	$C_9H_{16}O_4$	34	**nP**	10	**nP**	18237,64
Cholic acid	Steroids	$C_{24}H_{40}O_5$	34	**nP**	10	**nP**	2835,89
Cytidine	Pharmaceuticals	$C_9H_{13}N_3O_5$	23	**nP**	8	**nP**	7346,68
Deoxycholic acid	Steroids	$C_{24}H_{40}O_4$	27	**nP**	5	**nP**	4836,60
Deoxyuridine	Pharmaceuticals	$C_9H_{12}N_2O_5$	7	**nP**	8	**nP**	682,14
Diethylphthalate	Perfumes & PVC	$C_{12}H_{14}O_4$	23	**nP**	390	**vP**	174,24
Laurolactam	Nylon	$C_{12}H_{23}NO$	26	**nP**	8	**nP**	568,12
Laurylbetaine	Health care products	$C_{16}H_{33}NO_2$	23	**nP**	7	**nP**	1077,31
Leucine + Isoleucine	Supplements	$C_6H_{13}NO_2$	71	**nP**	15	**nP**	52,04
N,N Diethyltoluamide	Insect repellents	$C_{12}H_{17}NO$	71	**nP**	241	**vP**	38,55
Theophylline	Pharmaceuticals	$C_7H_8N_4O_2$	20	**nP**	4	**nP**	288,29
Triethylene glycol	Pharmaceuticals	$C_6H_{14}O_4$	23	**nP**	26	**nP**	43,42
Tyrosine	Supplements	$C_9H_{11}NO_3$	71	**nP**	1072	**vP**	28,83
Xantine	Pharmaceuticals	$C_5H_4N_4O_2$	16	**nP**	4	**nP**	1334137,33

4.4 Sonozone: pilot-scale

The PP reactor must be tested and compared to the laboratory-scale results. Since it was demonstrated at the laboratory-scale that the US pretreatment may prompt the radical generation, enhancing the ozone transfer rate into the liquid matrix, the efficiency of the process was higher than the single application of these AOPs. The same has to be tested with the continuous pilot-scale hybrid treatment.

4.4.1 Ozone concentrations

The two series within the Fig. 4-w should be intended as follow: the “single inlet” is the process performed only with the inlet ozone at the bottom of column “a”, instead the “double inlet” includes the contemporary ozone inflow from both the columns “a” and “b”. Columns “a” and “b” were presented in Fig. 3-h.

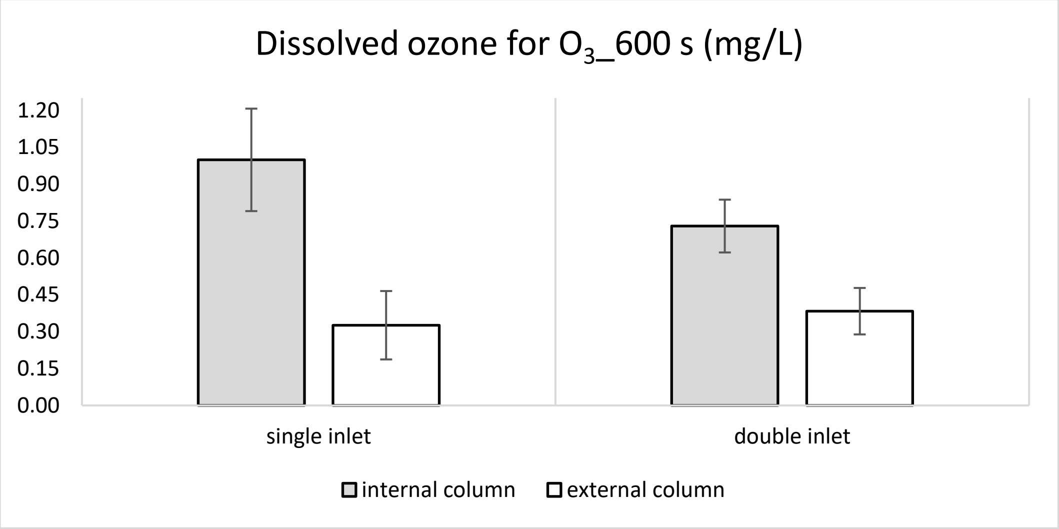

Figure 4-w: Dissolved ozone in the PP reactor

The dissolved ozone concentration in tap water was measured with the above-mentioned Indigo method (4500-O_3 B, Standard methods). The evaluation was applied in both the internal (“a”) and external (“b”) columns, sampling a 100 mL volume every 10 minutes of ozonation.

4.5 Wastewater reuse: the circular economy concept

It was demonstrated that WW is an extremely valuable source of water and nutrients, and thus it must be used in a sustainable way. Nowadays, water scarcity is a burden in several areas and non-stopping spreading worldwide, forcing the countries and international organization to push towards the reuse and recovery of the resources. The European Commission, in 2015, adopted a plan called “Closing the loop—a European Union (EU) action plan for the Circular Economy” concerning the circular economy concept for the closure of the cycle, started with water withdrawal from nature [123]. This strategy aimed to promote the reclamation of WW through legislative proposals, such as the recent regulation (EU) 2020/741. The water resource recovery recently became a prior sector of investments [124]. Above all, RWW for agricultural irrigation is driving the circular economy concept through the direct application of water and nutrients on crops. The outstanding result may convert the exploiting of resources in their controlled reuse. However, the reuse has to be constantly safe, ensuring a high level of protection for the human and animal health and for the environment. Moreover, the United Nations 2030 Agenda for Sustainable Development defined the SDG6 that focuses particularly on water sanitation [125]. Other remarkable goals, not directly focused on water, are strongly related to the circular economy concept: SDG 11 (make cities and human settlements sustainable and resilient) and 12 (ensure sustainable consumption and production patterns). The combination of these strategies may transform the consumption habits of the European population into a climate neutral way of life, reducing the environmental impacts. EU countries were additionally forced to upgrade their conception of waste, after the Waste Framework Directive 2008/98/EC. The establishment of a hierarchy was an innovation that set new necessary measures to recover, reuse and recycle waste. The prerogative was the prevention, followed by the preparing for reuse, the recycling, other recovers and finally the disposal. The hierarchy must be respect in order, clearly imposing waste disposal exclusively as a last resort [126]. Nutrients and altered water must be treated as waste, adopting the passages as explained. The sustainable development of waste management was a visionary concept toward the "zero waste" [127]. For instance, nitrogen has been classically seen as a hazardous component that has to be removed in the activated sludge along the WWTP line. The legislation often requires total N removal in WWTP effluents to very low limits (< 15 mg/L), implying additional energy demands on conventional, carbon-removing treatment processes [62]. The nitrogen contained in industrial fertilizers is produced through the Haber-Bosch process by fixing atmospheric N_2. However, this is an energetically and economically expensive process, requiring 19,3 kWh/kg N produced [128]. Moreover, phosphorus is even more scarce than nitrogen, and is never found free (in its elemental form) in nature but exclusively in phosphorus-containing minerals. Phosphorus resources are distributed unevenly in the world and are fast depleting, boosting the need for efficient recovery technologies from high-loaded streams, such as wastewater effluents. The legislation sets the P threshold as < 2 mg/L and its recover rate is higher from the solid phase (sludge) than the liquid one (WW), due to higher concentrations in the former. P recovery from a WWTP effluent will effectively save the 2,11 kWh/kg P used during the mineral P production process [27]. Exploiting WWTPs flexibility by modifying treatment sequences could produce nutrient-rich effluents, suitable for fertigation. For the extent of demonstrating a meaningful possibility of reusing WW for agricultural irrigation, the following case study was performed. The nutrients will be considered as a waste exclusively whether their concentration after treatment is higher than the plant needs.

4.6 Fertigation case study

In this section, a logical methodology to evaluate the techno-economic feasibility of WW fertigation practices is outlined and applied to a relevant Italian case-study. Hydrological and economic advantages of fertigation are determined considering water and fertilizer requirements of local crops and are compared to mineral fertilizers and agricultural water consumption under traditional practices.

4.6.1 Methodological framework

The methodological protocol to assess the feasibility of effluents fertigation considers effluent flowrate and quality, target crops requirements, and the assessment of water and nutrients that could be fulfilled from RWW. Mass balances are drawn to avoid overfertilization and, finally, a simplified economic assessment is implemented to evaluate fertigation sustainability and efficiency over traditional practices.

The irrigation water volumes that should be supplied are determined based on monthly water balances. Turc's equation [129] is applied to determine the effective rainfall (R, L/s ha) from meteorological data. In a simplified approach, the crop evapotranspiration (ET, L/s ha) can be considered equal to irrigation water requirement. The net irrigation requirement (I, L/s ha) can be calculated monthly through *Eq. 12*:

$$I = (ET - R)/E \qquad (Eq.\ 12)$$

In *Eq. 12*, E is the overall irrigation efficiency, obtained by multiplying irrigation system efficiency (E_d), water distribution efficiency from source to fields (E_t), and application efficiency (E_a) (*Eq. 13*), function of site's paedology.

$$E = E_t E_a E_d \qquad (Eq.\ 13)$$

The difference between ET and R represents the net water amount required for crop needs; this is used to estimate the necessary fertigation water volumes. N and P are considered as the main nutrients necessary for crops growth, while potassium was not included in the analysis since it is not usually measured in WWTP effluents. Specific crop nutrient requirements (kg/ha yr) are used to draw mass balances, considering applied water volumes and concentrations therein, together with fertilizer use efficiency. If fertigation supplied nutrient mass is lower than crop requirements, mineral fertilizers should be added; in the opposite case, fertigation limitation ensues, implying reduction of applied RWW, which should be complemented with supply from other sources.

A simplified economic evaluation is then performed, considering the cost of mineral fertilizers (ammonium nitrate or urea for N supply, triple super phosphate (TSP) for P supply), together with their use efficiency. Fertilizers' use efficiency varies according to fertilization practices, being lower in direct application to soils and higher in drip fertigation [130]. Concerning water saving, the cost of agricultural water supply is considered.

4.6.2 Case study application

This methodology was applied to from an urban WWTP with potentiality of 62.500 P.E. The location is an agricultural area where maize, rice, soybean, and vineyards are common crops. The WWTP employs an advanced treatment process train (pretreatment, primary clarification, enhanced biological phosphorous removal with nitrification/denitrification, final clarification, disinfection). Two years WWTP operating data (flowrate, influent and effluent concentrations) were considered. The mean influent flowrate was (26,54 ± 7,62) x 10^3 m^3/d, while mean available flowrates during the irrigation season (April - September) are reported in Table 4-11. Local meteorological data [131] were used to calculate the average effective rainfall (R) (*Eq. 12*), in the preceding 5 year period (2016 - 2020).

Table 4-11: Minimum, average, and maximum WWTP flowrates in the irrigation period (April - September).

Month	$Q_{average}$ x 10^3 (m^3/d)	Q_{min} x 10^3 (m^3/d)	Q_{max} x 10^3 (m^3/d)
April	33,168	22,161	39,275
May	45,179	22,551	77,676
June	23,969	14,735	27,400
July	19,238	17,986	21,457
August	20,541	17,761	23,047
September	23,797	16,550	39,728

Two different scenarios were simulated for the techno-economic analysis (Table 4-12): (a) actual nutrient concentration in the effluent, and (b) maximum nutrient concentrations allowed by current Italian regulations (Table 2-6). Actual nutrients effluent concentrations, expressed as TN and TP, were significantly lower than reuse limits. The application of the sonozone technique could be considered as included in the second scenario, where the maximum allowed concentration is recovered. When the N and P concentrations in the reclaimed WW exceed the reuse thresholds, some dilution may be required to fit all the required parameters.

Although K was not considered herein, it was however reported that its concentration in reused municipal effluents could provide up to 130 kg/ha to crops, higher than usual fertilization dosages (30 - 70 kg/ha) [132], and thus could be considered nonlimiting. Fertilizer use efficiency was respectively 40% for N and 20% for P for mineral fertilization, while it was assumed to be 95% and 45%, respectively, for fertigation, since this practice ensures that nutrients are supplied precisely at the area of most intensive root activity according to the specific crop requirements [130].

Concerning the economic analysis, two positive terms (savings) are considered as a consequence of reclamation: firstly, the reduction in mineral fertilizers use and then the reduction in primary water consumption. It was initially assumed that no fee is paid by farmers for reclaimed water. However, additional positive inputs to supply utilities' economic balance could be included if farmers would be willing to pay for RWW the same price charged for traditional irrigation water. Local databases of agricultural and reclamation associations were examined to estimate both mineral fertilizer costs [133] and irrigation water cost [134].

Table 4-12: Physico-chemical parameters considered in the two investigated scenarios

Case	TSS (mg/L)	BOD_5 (mg/L)	COD (mg/L)	TP (mg/L)	TN (mg/L)	Ammonia (mg/L)
Case 1: WWTP effluent	5,88 ± 6,17	7,79 ± 3,20	31,25 ± 12,27	0,86 ± 0,51	6,89 ± 2,80	0,30 ± 0,58
Case 2: Limits for wastewater agricultural reuse	10	20	100	2	15	2

The investigated crops significantly differ in terms of nutrient and water requirements throughout the irrigation period (Table 4-13): maize, soybean and vineyards require irrigation from April to August, while rice requires watering from May to September. The maximum water demand is typically encountered in July, even though vines showed an almost continuous demand. Water requirements were calculated for humid subtropical climate (Cfa in the Koeppen classification) and medium-textured soil conditions. As for nutrients, vines need the lowest N and P amounts, while maize and rice demand significantly higher fertilization. Rice requires higher P compared to other crops.

Table 4-13: Water and nutrient requirements for the considered crops.

Crop	**Water requirement (L/s ha)**						**Nutrient requirement (kg/ha yr)**			**Reference**
	April	May	June	July	Aug.	Sept.	N	P	K	
Maize	0,42	0,53	0,53	0,65	0,53	0	135	60	180	[135], [136]
Soybean	0	0,45	0,52	0,65	0,52	0	20	40	20	[130], [135]
Rice	0	0,69	0,69	0,69	0,56	0,48	162	120	72	[135], [137]
Grapevine	0,64	0,45	0,45	0,45	0,45	0	10	10	10	[138]

Total irrigation efficiency (E) was calculated, assuming E_t and E_a values of 0,90 and 0,95, respectively (*Eq. 13*), corresponding to water conveyance by concrete-lines open channels from source to distribution point, and medium-textured soil structure. Four different final water distribution systems were considered, namely: surface irrigation (E_d = 0,60), drip irrigation (E_d = 0,70), sprinkler (E_d = 0,75) and hose reel (E_d = 0,90).

In the lowest efficiency scenario (surface irrigation) (Table 4-14), overall water requirements are highest for rice (9,283 x 10^3 m^3/month ha), followed by maize (10,6% less than rice), grapes (23,2% less than rice) and soybean (29,0% less than rice). According to this simplified approach, water demand is inversely proportional to irrigation systems' efficiency (Table 4-14).

Table 4-14: Specific irrigation requirements I (m^3/month ha) of the investigated crops using irrigation systems with different efficiency E_d.

Water distribution systems	Months	I (m^3/month ha)			
		Maize	Soybean	Rice	Grapevine
Surface irrigation	April	1219	0	0	2330
	May	1386	968	2222	968
	June	1265	1214	2073	861
	July	2777	2777	2986	1733
	August	1652	1599	1808	1234
	September	0	0	194	0
Sprinkler	April	975	0	0	1864
	May	1109	775	1777	775
	June	1012	971	1659	688
	July	2221	2221	2389	1386
	August	1321	1279	1447	987
	September	0	0	155	0
Drip irrigation	April	1045	0	0	1997
	May	1188	830	1904	830
	June	1084	1041	1777	738
	July	2380	2380	2559	1485
	August	1416	1371	1550	1058
	September	0	0	166	0
Hose reel	April	812	0	0	1554
	May	924	646	1481	646
	June	843	810	1382	574
	July	1851	1851	1990	1155
	August	1101	1066	1205	823
	September	0	0	129	0

The amount of nutrients supplied by RWW, based on calculated minimum irrigation volumes, are shown in Figure 4-x. According to this approach, E_d influences just the total water requirement, since nutrient calculations are based on net irrigation requirements (I), depending only on crops characteristics. In the case of soybean and vines, fertigation may lead to N oversupply. Therefore, water volumes were recalculated to limit N supply to crop requirements. As a consequence of fertigation, significant reduction in mineral N fertilizer dosage could thus be achieved, while P shows very limited supply by RWW. In the second scenario (RWW nutrient concentrations equal to agricultural reuse standards), relevant reduction in mineral N addition (> 40%) could be achieved for all crops, while P supply by RWW was again low. Proper tailoring of WW treatment could lead to improved effluent characteristics for specific crop requirements under a fit-for-purpose approach. In the specific example, lower P removal in the WWTP would provide a more balanced N:P ratio for fertigation, still assuring discharge limits fulfilment. However, the lower P use efficiency, compared to N, remains a limiting factor for fertigation practices, and consequently some mineral fertilizer addition appears unavoidable in most cases.

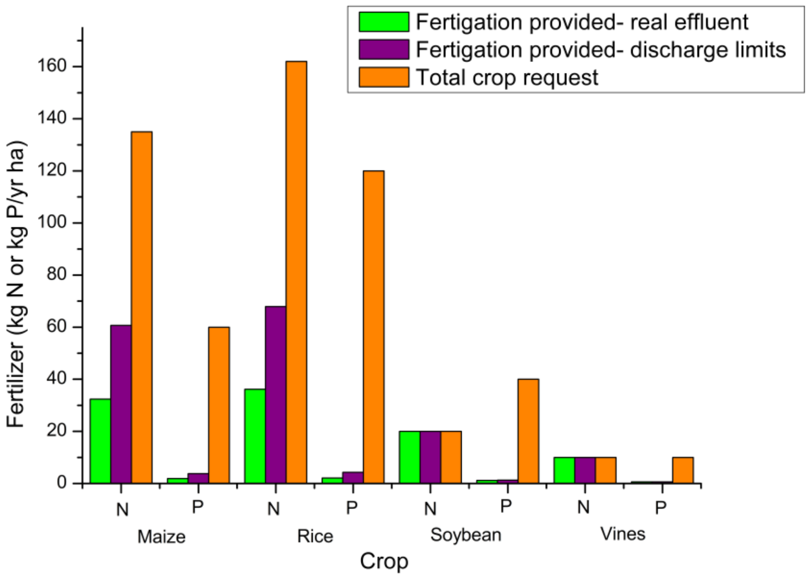

Figure 4-x: Fertilizers provided by fertigation (kg/ha yr) in comparison with total crop demand in the investigated scenarios.

Results of the economic analysis are summarized in Figure 4-y and Table 4-15. Relevant savings (up to > 40%) in mineral fertilizers could be obtained through fertigation (Figure 4-y). Vines proved to be the most favored crop. Lower benefits (25%) were calculated for soybean, with maize and rice showing worse performances (respectively 15% and 11% savings).

For all crops, water savings were remarkably higher than mineral fertilizer's, due to the huge water volumes that must be supplied, especially for maize and rice (Table 4-15). RWW with nutrient concentration up to regulatory limits (scenario 2) would reduce water savings for soybean and grapevine, because all N demand is already covered by actual RWW (scenario 1), and P supply is limited by its low use efficiency. The economic balance could be closed if farmers were charged by WWTPs a tariff equal to that currently paid to agricultural irrigation consortia (0,2 €/m^3 in the current analysis): total cost for wastewater reclamation (including capital and operating costs), in addition to wastewater treatment cost, were estimated in the range of 0,25 - 0,50 €/m^3 in Italy [139]. Thus, such tariff would cover the additional costs for water utilities; public subsidies or incentives could further improve the balance.

Table 4-15: Water, fertilizers, and total economic savings (€/ha yr) for scenarios 1) and 2).

	Scenario 1 (actual effluent)			**Scenario 2 (legislation limits)**		
Crop	Water savings (€/ha yr)	Fertilizer savings (€/ha yr)	**Total savings (€/ha yr)**	Water savings (€/ha yr)	Fertilizer savings (€/ha yr)	**Total savings (€/ha yr)**
Maize	851	76	927	851	144	995
Rice	952	85	1037	952	161	1113
Soybean	526	47	573	281	47	328
Grapevine	263	24	287	140	24	164

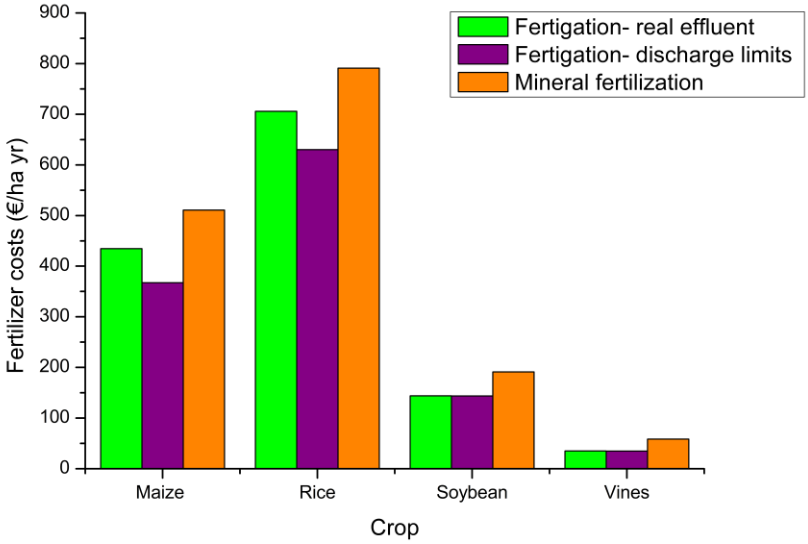

Figure 4-y: Simulated yearly fertilizer costs (€/ha yr) for the two investigated scenarios versus baseline conditions (mineral fertilization).

4.7 Social acceptance

Demonstrating the importance of the fertigation practice towards water and nutrient recovery from WWTP effluents and highlighting its relevance for the circular economy perspective is not sufficient. The obstruction against its application on the everyday activities due to the sociocultural acceptance could be a limiting factor for the stable implementation of this practice. WW reuse feasibility is strongly linked to stakeholders' acceptance, from farmers to consumers, which may be independent of the scientific evidence supporting the related benefits [140]. Public dissemination approaches are often used to investigate and encourage stakeholders' willingness to adopt such schemes. A Brazilian study shown relations between the citizen's educational level and the acceptance of the reuse practice. The perception of water as abundant made them believe that no needs of reuse are necessary, supporting the culture of waste [141]. Moreover, marked differences are related to the gender, ethnicity, age, sector of employment, religion and income. The fear against the pathogenic contamination and the chemicals in WW are the most limiting thoughts. Nonetheless, the answers reported an opening towards the acceptance of reuses in industrial applications (firefighting, toilet flushing, street cleaning), non-food agriculture (groundwater recharge, forest irrigation, sport fields, industrial crop irrigation) and partial household applications (toilet flush). On the other hand, all the remained household employments and edible crop agriculture utilizations are currently seen as unacceptable. Low human contact applications are admissible, while sectors involving direct and indirect consumption or skin contact are less accepted [142], [143].

Generally, enhanced information availability and transparency increase farmers' willingness to adopt WW reclamation practices. Moreover, the perception of a helpfulness towards the reuse and a favorable economic background, with dedicated incentives, coupled with a positive political attitude, are important aspects to consider [140], [144]. Young farmers with higher education levels generally demonstrate more interest in WW reuse schemes compared to other categories. Younger farmers showed stronger acceptance of nonconventional water sources, were interested in visiting WWTPs and understanding their operations, and exhibited higher trust on alternative supply [143], [145].

Nowadays, continued encouragement and incentivization of farmers and WWTP operators to widen RWW fertigation practices, by adopting suitable strategies under circular economy perspective, appears ever more necessary. Virtuous solutions addressing contingent factors such as water source availability and quality and appropriate crop selection should be promoted by agricultural agencies by providing exhaustive and transparent information to stakeholders and citizens. A combination of measures, including pollutant reduction at source, enhanced treatment focusing on persistent, bioaccumulative and toxic pollutants, careful agricultural practices and transparent information to stakeholders will be necessary to ensure safe and accepted widespread agricultural water reclamation schemes [146].

4.8 Chapter conclusions

The properties of a PEff from an urban WWTP (100.000 P.E.) were assessed before and after a hybrid AOP treatment, consisting of an ultrasonic pretreatment followed by an ozonation step. The results were presented in the form of percentage difference between the raw initial value and the output from the laboratory test. The final result, in terms of concentration, may be obtained multiplying the initial value (mg/L) to the respective percentage obtained after the treatment.

As concerns the numerical results, the ozone consumption increase was shown to demonstrate the US pretreatment efficiency towards a better dissolution of the gaseous phase in water. Moreover, using the Indigo colorimetric method a stable concentration of ozone in water was determined. The validation of the combined effect concerning the US and O_3 process on WW was performed on:

- Solids (TSS): potential in the reduction of the suspended part. with particle size distribution shift from 10^3 nm to 1-10 nm;
- Organic matter (TOC, tCOD, sCOD, UV_{254}): consistent with the solids result, the total COD was reduced but the shift in particles size generated an increase in the soluble COD concentration;
- Nutrients (TN, Ammonia, TP, K, Ca, Mg): a clear demonstration that the ozone treatment is suitable for effluent reuse, due to its propriety of maintaining the nutrient concentration inside the WW. Reduction percentages limited below 10% (TN, Ammonia, K) or 25% (TP);
- Microorganisms (E. coli, Enterococcus spp.): for ozone treatments longer than 60 s the common pathogenic concentration of an urban WWTP was almost completely removed (> 99,99%). Salmonella was absent in the plant influent;
- Micropollutants (Formaldehyde, Anionic surfactants): being well-known ozone DBPs, formaldehyde was assessed and verified to be highly removed. In the same way, sonozone is suitable for the removal of diffused urban WW pollutants, like anionic surfactants;
- Heavy metals: ICP analysis shown that the untreated and the best-treated samples had no differences in the HMs content. The ozone mineralization rate at the current concentrations was meaningless, however the initial HMs concentration was lower that the legislation thresholds.

Table 4-16 reports the sonozone removal capacity against the main characterization parameters for the 60 s treatment, single and combined. 60 s are reported as an example of a possible treatment time, where the enhanced efficiency of the sonozone process compared to the current initial conditions is maximized for shorter US durations and ozone applications, due to the higher removal percentages and the lower energy consumptions. The only O_3_60 was used as the reference (0% of remotion) and the combined process values are calculated as percentual differences. Negative values represent an augment in the concentration compared the initial value.

Table 4-16: Summary of the results obtained with O_3_60

60 s ozone treatment time							
-	**TSS**	**COD**	**TN**	**TP**	**Formaldehyde**	**Anionic surfactants**	**E. Coli**
Raw	0%	0%	0%	0%	0%	0%	0%
US 5 s	13%	3%	1%	30%	32%	0%	71%
US 10 s	30%	-10%	1%	20%	30%	5%	97%
US 30 s	49%	5%	1%	26%	65%	26%	99%

In order to give a thorough overview, a basic energy balance (kWh/m^3) and economic evaluation (€/m^3) was conducted as well.

Considering that one of the most critical aspects of RWW agricultural reuse is the pathogenic infection, particularly when human contact is observed with the crop or the soil, an in-depth microbial interpretation was performed. The sonozone was tested, through several US and O_3 durations, towards 4 different microbial strains. The results, expressing once again an enhanced abatement of the combined process compared to the single ozone treatment, were technically and economically meaningful.

Moreover, the ecotoxicological assessment set an important result towards the targeted agricultural reuse. In-silico evaluations of ecotoxicity, persistence and solubility in water remarkably supported the disinfectant capacity of the sonozone.

Testing the hybrid process at a laboratory-scale gave rise to an interest towards the scaling-up of the reactor (from 1 L to 50 L). The PP reactor was built and connected with all the other components. The hydraulic controlled flow and the gas dissolution rate were assessed. Longer application times compared to the laboratory tests could be needed, however the final assessment of the PP will be performed in future applications.

Even though the exposed techno-economic assessment appeared to be relevant for the study, a significant aspect to be further investigated is the sustainability of the reuse and the social acceptance. Nowadays, the circular economy concept is one of the most crucial, the validation of the fertigation practice must consider it. Arising out of the case study, it was demonstrated that the RWW agricultural reuse may be suitable for water and nutrient reclamation, related to an economic, waste-reduction, water-saving and pollution control point of view. Citizens and stakeholders are not still aware about the fertigation potentialities; educational, cultural and political obstacles are slowing down the possible implementation in the everyday uses.

5 CONCLUSIONS

The final purpose of this study was the reuse of the nutrients contained inside the wastewater: ozone properties are well-known not to reduce the nitrogen-phosphate content, and this is the reason why this process was chosen. Ultrasounds could enhance the subsequent ozone disinfection efficiency; sonication used as a pre-treatment could increase the amounts of highly active ·OH radical formation, responsible for pollutant degradation. The nutrients and water recovery established a new circular economy approach contributing to the challenge of raw materials depletion. The reclaimed wastewater fertigation could provide a suitable substitute to a traditional irrigation practice, compensating for freshwater shortages in agriculture and reducing mineral fertilizers application, with significant environmental and economic benefits.

The combination of low-frequency ultrasound and ozonation tests was designed and performed for the primary effluent recovery for agricultural purposes. The evaluation of the hybrid technology demonstrated a remarkable removal efficiency towards the solid fraction and the organic matter. The microorganisms (fecal pathogens) and micropollutants (formaldehyde and anionic surfactants) treatment through the sonozone process highlighted a significant abatement, able to reduce their concentrations below the legislative thresholds. During the US-O_3 combined application, the nutrient load, measured as total nitrogen and total phosphate, was well-maintained. Consequently, a high potential value for the agricultural reuse of the treated primary effluent was highlighted, with possible significant saving of chemical fertilizers.

Ultrasounds and ozone are well-known high-energy consuming processes compared the classic WWTPs biological treatments. A basic economic evaluation demonstrated that the sonozone technique requires a huge amount of energy. However, it is possible to consider that the application of a sonozone disinfection may result in the unnecessity of a classic activated sludge process, as well as of a tertiary wastewater disinfection, avoiding high chemical cost and energy for additional treatments. Furthermore, a nutrient recovery means that the mineral fertilization for N and P plant nourishment is reduced. Industrial processes, for nutrient production, and freshwater costs are significantly decreased. Besides, the regions where the water availability is scarce have unavoidably a higher freshwater price. Originating from these considerations, the sonozone process appeared to be economically applicable, especially in decentralized areas not served by public sewers and wastewater treatment plants. A localized application has to be supported with a pilot campaign to gather more data. Remote and decentralized areas may become the ideal place for the sonozone application when connected with the integration of solar energy, providing a sustainable way of producing its electric need.

The in-depth pathogen assessment was based on four different bacteria, after several distinct treatments. Singular and combined ultrasonic and ozone processes were tested to establish the reduction rates of *Salmonella Enteritidis*, *Escherichia coli*, *Enterococcus* spp. and *Pseudomonas* spp. Ultrasound alone resulted to be inadequate towards an efficient pathogen removal at our design conditions. The ozone employment resulted to fit for the hypothesized wastewater microbial removal, according to the desired reuse scheme. In particular, the observed removal rates after an ultrasonic pretreatment appeared to be constantly higher than the only ozone processes. Towards a comprehensive assessment of the hybrid process, an economic evaluation was conducted. The main conclusions arising from the data elaboration provide an overall satisfactory comeback. The comparisons between the energy consumptions revealed that, while operating through brief US pretreatment times, the sonozone resulted to be whichever a stronger treatment, economically convenient compared to the single ozonation. Notwithstanding an augment in the pathogen reduction rate, the employment of longer ultrasonic pretreatment times does not regularly correspond to a relevant money saving. Future experiments should be designed to the extent of testing even shorter pretreatment times and adopting a wider range of ozone subsequent dosages.

Several compounds were measured and used as an input for an in-silico ecotoxicological evaluation. Their behavior towards the sonozone oxidation was obtained as well. The results shown that some volatile aldehydes may be formed during the ozonation process, not being present in the raw mixture. The ecotoxicological tests on vertebrates, invertebrates and plants in addition to the soil and water persistence and water solubility, gave meaningful information regarding the duration needed for their removal and the toxicity level. However, the sonozone shown remarkable capacities in the removal of these resistant compounds. Further ecotoxicological assessment are required for an exhaustive estimation of the hazardous properties of the wastewater chemicals and the disinfection by-products.

From the laboratory tests it was concluded that low-frequency ultrasound pre-treatment, combined with ozonation, could be a useful process for primary effluent recovery for several purposes. The combined treatment showed a stable capacity of enhancing the ozone efficiency on the main wastewater compounds, maintaining the nutrients level. Further studies are expected to be planned and executed to evaluate system scale-up feasibility and the detailed effects of the most meaningful process parameters on final effluent toxicity. The dissolved ozone concentration was demonstrated to be consistent with the laboratory scale value and stable inside the pilot plant reactor. Further tests have to be performed for the pilot-scale wastewater treatment assessment and, additionally, the pressure inside the reactor may be controlled and advantageously increased to obtain a better ozone dissolution inside the wastewater. As a future work, the data coming from a robust pilot-scale analysis may be employed for a model-based simulation, optimization and techno-economic feasibility of the overall process.

Moreover, a techno-economic methodology was presented to assess agricultural fertigation sustainability. Results from its application to a real case-study showed that significant mineral fertilizers savings could be achieved on selected crops, particularly soybean and vines, which could be easily supplied with most of the required N. Low effluent concentration and limited use efficiency of P reduced the overall economic benefit in this case. Wastewater treatment could be adjusted to lower P removal targets in the “fit-for-purpose” concept, providing more available P to crops; however, higher cost reductions were obtained from water savings, rather than mineral fertilizers. In addition, a follow-up of the fertigation techno-economic case study could be related to consider the monthly needs of the crops, instead of their annual balance, and their yield reduction due to salinity, including new relevant crops. The proposed methodology could be improved by adding site-specific considerations concerning energy and GHG emissions of tailored fertigation strategies and evaluating changes in WWTP operations to provide the desired effluent quality. The methodology could be applied to any location, giving useful preliminary insight related to the feasibility of reclaimed wastewater fertigation schemes. Anyway, before the fertigation adoption, it is imperative to assess the correspondence between treated effluents availability and quality, not forgetting the irrigation demand. The presence/absence of suitable water distribution networks for effluents conveyance to the fields is also an important factor of choice, as well as the availability of appropriate water storage tanks to provide flexibility in fertigation planning. The collaboration between stakeholders, from water utilities to agricultural consortia and farmers, is a key point to achieve success. Punctual effluent monitoring and early warning on pollutants and pathogens could reduce the diffused skepticism that still undermines fertigation social acceptance. Compared to traditional fertilization, fertigation allows better nutrient use efficiency, improving plant uptake and nutrients availability in the root zone; however, long-term salinity and sodium monitoring is mandatory, due to high SAR levels in treated flows. Finally, risk management approaches, such as those proposed by EU Directive 2020/741, can allow early detection of other possible issues related to agronomic conditions. More importantly, wastewater fertigation should be evaluated under a circular economy perspective. Water discharge regulations do not always allow the complete fulfillment of crops nutrient requirements, needing high removal efficiency which may be in contrast with the full exploitation of wastewater-embedded resources and implying heavier energy and emissions footprint on WWTPs. A more flexible approach to effluents discharge could maximize fertigation impact without impairing environmental protection goals.

In conclusion, this combined sonozone treatment is an example of an innovative way of recovering wastewater and the meaningful compounds therein embedded, respecting the criteria defined by the circular economy perspective. From the considered legislative thresholds expressed for the non-edible crops, in order to maintain the nutrient concentrations below the given limits, a dilution of the treated primary effluent should be necessary in some cases.

6 LIST OF PUBLICATIONS

The current study is based on the work contained in the following papers, sort by date from oldest to newest:

1. Mainardis, M., Cecconet, D., **Moretti, A.**, Callegari, A., Goi, D., Freguia, S., & Capodaglio, A. G. (2022). Wastewater fertigation in agriculture: Issues and opportunities for improved water management and circular economy. Environmental Pollution, 296, 118755. https://doi.org/10.1016/j.envpol.2021.118755
2. **Moretti, A.**, Zanolla, A., & Goi, D. Combined Ultrasound-Ozone treatment for reutilization of a primary effluent, *In progress.*
3. **Moretti, A.**, Gover, E., Goi, D., & Marino, M. Kinetic modeling and cost-effectiveness of Salmonella Enteritidis, Escherichia coli, Enterococcus spp., and Pseudomonas spp. inactivation in wastewater through Advanced Oxidation Processes (AOPs), *Submitted*.

The author made the following contributes:

1. Investigation; Result analysis; Writing the original draft; Visualization.
2. Conceptualization of the research; Methodology; Result analysis; Investigation; Data Curation; Writing the original draft; Visualization.
3. Conceptualization of the research; Methodology; Result analysis; Investigation; Data Curation; Writing the original draft; Visualization.

Publications not included in the thesis completed during the PhD:

1. Campana, P. E., Mainardis, M., **Moretti, A.**, & Cottes, M. (2021). 100% renewable wastewater treatment plants: Techno-economic assessment using a modelling and optimization approach. Energy Conversion and Management, 239, 114214. https://doi.org/10.1016/j.enconman.2021.114214
2. Mainardis, M., Buttazzoni, M., Cottes, M., **Moretti, A.**, & Goi, D. (2021). Respirometry tests in wastewater treatment: Why and how? A critical review. Science of the Total Environment, 793, 148607. https://doi.org/10.1016/j.scitotenv.2021.148607

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