Chelating agents supported solar photo-Fenton and sunlight/H_2O_2 processes for pharmaceuticals removal and resistant pathogens inactivation in tertiary treatment for urban wastewater reuse.

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ABSTRACT

In this work, Fe^{3+}-iminodisuccinic acid (Fe:IDS) based solar photo Fenton (SPF) was investigated in tertiary treatment of real urban wastewater and compared to Fe^{3+}-ethylenediamine-N,N'-disuccinic acid (Fe:EDDS) for the first time. Three pharmaceuticals (PCs) (sulfamethoxazole, carbamazepine and trimethoprim) and four pathogens (*Escherichia coli*, somatic and F-plus coliphages, *Clostridium perfringens*, consistently with the new EU regulation for wastewater reuse (2020/741)), were chosen as target pollutants. The best operating conditions resulted: H_2O_2=50 mg L^{-1}; Fe=0.1 mM; Fe:L=1:1; alkalinity=75 mg L^{-1} for both Fe:Ligand (Fe:L) based SPF processes. SPF with Fe:EDDS was more effective in PCs removal (80%, 10 kJ L^{-1}) than the SPF with Fe:IDS (58%). On the contrary, Fe:IDS resulted more effective (4.3 log inactivation for *E. coli*) than Fe:EDDS (1.9 log) in pathogens inactivation. Fe:L based SPF was subsequently coupled to sunlight/H_2O_2. Interestingly, while its combination with Fe:EDDS based SPF slightly increased disinfectant efficacy (2.3 vs 1.9 log inactivation for *E. coli*), the combination with Fe:IDS showed an opposite effect (3.4 vs 4.3 log reduction). Such results, make the use of Fe:IDS an attractive option in SPF tertiary treatment for urban wastewater reuse.

Keywords: advanced oxidation processes; contaminants of emerging concern; natural pH; photo-Fenton like; viruses.

ENVIRONMENTAL IMPLICATION STATEMENT

Pharmaceuticals and pathogens are considered as hazardous chemical and biological pollutants and are among the main environmental and health challenges in tertiary treatment of urban wastewater for reuse. In particular, pharmaceuticals have been recently included in the new EU wastewater reuse regulation as hazardous pollutants to take into account within the risk management plan. Accordingly, in this work a sustainable solution for small wastewater treatment plants coupling sunlight/H_2O_2 and solar photo Fenton (SPF) with a new chelating agent was compared to a consolidated chelating agent to effectively remove such pollutants and operate SPF at neutral pH.

1. Introduction

Climate change, pollution, population growth and conflicts are among the main factors causing high water-stress across the globe (Wang et al., 2021). Preserving water quality and ensuring its availability is one of the most serious challenges for several countries in the world (Kummu et al., 2016, UN World Water Development Report 2020). Indeed, around four billion of people face severe water scarcity every day (Mekonnen et al., 2016). In particular, climate change is causing serious problems even in geographical areas only marginally or not affected by water scarcity until a few years ago. In this context, wastewater reuse represents an excellent strategy to overcome the water crisis (Scheierling et al., 2011; Villarín and Merel, 2020). At the same time, caution should be taken in applying this practice, since improper treatments can lead to dramatic consequences (Fatta-Kassinos et al., 2011). While in low-income countries, the main risk factor in the practice of wastewater reuse is the presence of pathogenic microorganisms, in high-income countries contaminants of emerging concern (CECs) must also be considered (Fatta-Kassinos and Costas, 2013; Pastorino and Ginebreda, 2021). CECs include active ingredients of pharmaceuticals (PCs), pesticides, personal care products etc., that although occurring at low concentrations (in the range ng L^{-1} - µg L^{-1}) in water and wastewater, can accumulate in the environment resulting in chronic toxic effects (Christou et al., 2017; Hossain and Roy, 2018). Moreover, among PCs, antibiotics are of additional concern because can trigger antibiotic resistance, which represents a serious threat to human health (Cacace et al., 2019). Unfortunately, commonly employed processes in urban wastewater treatment plants (WTPs) are scarcely efficient in the removal of CECs and the scientific community is committed in investigating possible solutions for an effective and sustainable tertiary treatment (Rizzo et al., 2019). Advanced oxidation processes (AOPs), relying upon the build-up of highly reactive oxygen species (ROS), are among the most investigated solutions for tertiary treatment of urban wastewater (Comninellis et al., 2008; Deng et al., 2015; Rizzo, 2022). Particularly, solar photo-Fenton (SPF) like process, implementing chelating agents to effectively operate the

process at neutral pH, it allows to overcome the limitation of the conventional process which is effective at acidic pH and it has been attracting increasing interest as possible solution in small WTPs (Prieto-Rodríguez et al., 2013; De la Obra et al., 2017; Fiorentino et al., 2019; Oller and Malato, 2021; Gualda-Alonso et al., 2022). Such an interest is expected to further increase in the next years because the growing energy cost, water scarcity and sustainability policies are driving toward the implementation of sustainable green solutions for wastewater treatment and reuse. The presence of chelating agents in SPF like process delays the precipitation of iron at neutral pH, allowing an effective degradation of the target pollutants (Clarizia et al., 2017). Polycarboxylic and aminopolycarboxylic acids have been used as ligands for iron species (Baba et al., 2015; Prete et al., 2021) and ethylenediamine-N,N′-disuccinic acid (EDDS) has been successfully investigated in the degradation of CECs by SPF in secondary treated urban wastewater (Miralles-Cuevas et al., 2019; Maniakova et al., 2020). However, chelating agent supported SPF has still some drawbacks: (i) EDDS is quite expensive, making the process not yet competitive compared to conventional SPF at acidic pH (Sánchez Pérez et al., 2020); (ii) while Fe:EDDS supported SPF can provide a higher removal of CECs compared to sunlight/H_2O_2, it is less effective in bacteria inactivation (Maniakova et al., 2021a).

Iminodisuccinic acid (IDS), a biodegradable ligand (Schowanek et al., 1997; Cokesa et al., 2004), has been recently investigated as a chelating agent in water treatment (Fiorentino et al., 2021; Faggiano et al., 2022) and disinfection (Fiorentino et al., 2018), making the potential of Fe:IDS complex in tertiary treatment of urban wastewater by SPF worthy of investigation.

To speed up the possible application of SPF at full scale, in particular as tertiary treatment in wastewater reuse applications, tests under realistic conditions using real wastewater, different CECs and microorganisms should be implemented (Rizzo et al., 2020). The regulation of CECs in wastewater reuse has been widely debated (Rizzo et al., 2018; Deviller et al., 2020) before being introduced into the European regulation (2020/741). Accordingly, the relevant stakeholders are asked to take the occurrence of CECs into account, through a risk assessment plan, when authorizing reuse

of WTPs effluents. Moreover, the validation control, by including really stringent targets for pathogens inactivation (namely, $\log_{10}$ reductions ≥ 5.0 for *Escherichia coli*, ≥ 6.0 for different types of coliphages and ≥ 4.0 for *Clostridium perfringens*), make the identification of effective and sustainable tertiary treatment methods even increasingly challenging.

In this work, SPF process with Fe:IDS complex was investigated for the first time as tertiary treatment of urban wastewater at natural pH and compared to SPF with a consolidated complex, namely Fe:EDDS. Moreover, SPF was also compared to sunlight/H_2O_2 and to their combination through a sequential treatment approach in the effort to maximize both PCs removal and pathogens inactivation. Solar driven tests were carried at pilot scale in raceway pond reactors (RPRs), which are considered a cheaper solution compared to compound parabolic collectors-based reactors (Carra et al., 2014). The target PCs (sulfamethoxazole (SMX), trimethoprim (TMP) and carbamazepine (CBZ)) were selected as a result of a compromise among different factors: (i) their occurrence in urban wastewater and refractoriness to conventional wastewater treatment, (ii) representativeness of relevant pharmaceutical families/functions and (iii) relevance for crop uptake (Krzeminski et al., 2019; Christou et al., 2019). Different concentrations of Fe, Fe:L ratios and alkalinity values were investigated in terms of pharmaceuticals (PCs) removal. As the operating conditions were optimized, the disinfection capacity was investigated on different pathogens including *E. coli*, as indicators of pathogenic bacteria, coliphages as indicators of pathogenic viruses and *C. perfringens* as indicators of protozoa, consistently with the approach used in the EU regulation on wastewater reuse.

2. Materials and methods

2.1 Chemicals and reagents

High-purity grade (>99%) SMX, CBZ and TMP were sourced from Sigma Aldrich. Catalase from bovine liver, EDDS water solution (35%, w/v), Titanium (IV) Oxysulfate (99.99%), 1,10-phenanthroline (99%), ammonium acetate (98%) and acetic acid (99%) were purchased by Sigma

Aldrich. An Arium Mini Deionizer (Sartorius) produced deionized water employed in the experiments. $FeCl_3 \cdot 7H_2O$ (98%) and H_2O_2 (35%, w/v) were purchased from Carlo Erba Reagents. Acetonitrile (ACN) (LC-MS grade), formic acid (FA) (LC-MS grade) and H_2O (LC-MS grade) were purchased from Honeywell. Nalidixic acid, Indoxyl-B-D-glucoside, Polymyxin B Sulfate, D-Cycloserine, Phenolphtalein Diphosphate Tetrasodium salt were purchased from VWR. Tryptone bile X-glucoronide (TBX) Agar, Nutrient Brooth, Kovacs Reactant, Agar, m-CP Agar, *E. coli* ATCC 13706 and ATCC 15597 strains, Tryptone and Tryptone Soy Agar were purchased from Biolife. Ampicillin Sodium Salt (>99%), Magnesium Sulfate Heptahydrate (≥ 99.5%), Sodium Chloride (>99%), Sodium Dihydrogen Phosphate (≥ 99%), Potassium Phosphate dibasic (≥98%), EDDS and Ammonia solution (28%) were purchased by Sigma Aldrich. Anaerobic jars (2.5 L volume) were purchased from Oxoid. IDS was purchased by Glentham Life Sciences.

2.2 Wastewater characteristics

Secondary wastewater samples were collected from a WTP located in the province of Salerno, Italy (650000 equivalents inhabitants) implementing a conventional activated sludge process as biological treatment. Chemical and microbiological characteristics of the collected secondary effluent are reported in Table 1.

Table 1. Chemical and Microbiological Characteristics of the secondary wastewater.

Parameter	Mean value
pH	7.2 ± 0.1
COD (mg L^{-1})[2]	11 ± 2
BOD_5 (mg L^{-1})[2]	2 ± 1
Alkalinity (HCO_3^-) (mg L^{-1})	330 ± 10
Total Suspended Solids (mg L^{-1})	6 ±1

Turbidity (NTU)	0.47 ± 0.05
Total Nitrogen (mg L^{-1})	3 ± 0.2
Chlorides (mg L^{-1})	126 ± 11
NO_3^--N (mg L^{-1})	2 ± 0.2
NO_2^--N (mg L^{-1})	nd
***E. coli* (CFU[1] 100 mL^{-1})**	(2.9 ± 0.2) x 10^4
Coliphages (PFU[3] 100 mL^{-1})	(1.1 ± 0.1) x 10^4
***C. perfringens* (CFU[1] 100 mL^{-1})**	(1.0 ± 0.1) x 10^3

[1]CFU: colony forming units. [2] COD and BOD_5 values in Table 1 are lower of those generally reported in literature, because of the low organic load factor of the WTP. [3]PFU: plaques forming units.

2.3 Solar driven tests in RPR

2.3.1 Experimental set-up

Experiments in RPR (90 cm x 45 cm, 15 cm liquid depth and a working volume of 15 L) were carried out under natural solar irradiation at University of Salerno, Fisciano Campus, Southern Italy (40°76′N and 14°79′W), in sunny days during summer, from 11:00 to 14:00 local time, in order to exploit an approximatively constant solar radiance in the range 40-60 W m^{-2}. Irradiances varying in this range are not a limiting factor for SPF process in RPR (Cabrera-Reina et al., 2021). The RPR is equipped with a paddle wheel connected to an engine (with variable frequency, in the experiments it was set to 18 rpm) to facilitate the mixing of the chemical compounds (Fe complex, H_2O_2) in the aqueous solution. The total exposure for each experiment was 180 min. Irradiance spectra were measured in the range 280-400 nm by a radiometer (BLACK-Comet Stellar Net UV-VIS, StellarNet, Tampa, FL, USA) mounted on a horizontal platform. In PCs degradation tests, SMX, CBZ and TMP were spiked in the wastewater samples at an initial concentration of 0.2 mg L^{-1} each one (7.5 mL of stock solution). In disinfection experiments the effect on the indigenous bacteria population was evaluated. The

results were plotted as a function of the accumulated UV energy per unit of treated volume (Q_{UV}, kJ L^{-1}) (Malato et al., 2003):

$$Q_{UV,n} = Q_{UV,n-1} + \Delta t_n \cdot \overline{UV}_{G,n} \cdot \frac{A_r}{V_t} \quad \text{(Eq. 1)}$$

In the above equation, V_t is the total volume of water (L). Q_{UV} represents the UV energy per treated volume between samples *n* and *n-1* , $\overline{UV}_{G,n}$(W m^{-2}) represents the average UV radiation measured, Δt_n and A_r are the experimental time between samples and the illuminated area (m^2), respectively.

Different Iron:Ligand (Fe:L) ratios were investigated while an initial concentration of 50 mg L^{-1} (1.47 mM) of H_2O_2 was used, according to previous works (De La Cruz et al., 2013; Fiorentino et al., 2019). Sunlight/H_2O_2 experiments were performed with the same concentration of H_2O_2. Sequential treatment tests combining sunlight/H_2O_2 and SPF processes were performed starting with the sunlight/H_2O_2 process for 60 min and continuing with SPF for a total time of 180 min.

After adding the treated secondary wastewater sample in the RPR, H_2O_2 was added in one step and the aqueous solution was stirred for 2 min at 36 rpm. Subsequently, the iron complex (Fe:IDS or Fe:EDDS) was added and recirculated. In the PCs degradation tests, the contaminants (TMP, CBZ, SMX; 0.2 mg L^{-1}) were added at the beginning before adding the oxidant (H_2O_2). The resulting solution was mixed for 2 min before removing the cover from the RPR. Water temperature was monitored during the experiments and was always under 40 °C (30-38°C), therefore no significant temperature effect on bacteria inactivation was expected (O'Dowd and Pillai, 2020). For PCs removal, sampling times were 15, 30, 45, 60, 90, 120 and 180 min. For bacteria inactivation, sampling times were 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min.

2.3.2 *Preparation of Fe:L complexes and PCs stock solution*

Fe:L complexes were prepared by dissolving the appropriate amount of $FeCl_3 \cdot 7H_2O$ in deionized water and adding the desired amount of freshly prepared chelating agent solution (EDDS and IDS,

184 respectively) and the mixture was vigorously stirred for 10 min under dark, to avoid the instability of
185 the complex. In the case of Fe:EDDS complex, one drop of H_2SO_4 solution (95%) was also added to
186 improve the solubility of the complex. PCs stock solution was prepared dissolving PCs (16 mg each
187 one) in 40 mL aqueous solution (90/10 H_2O/EtOH).
188

189 ***2.3.3 Alkalinity effect***

190 To evaluate the effect of alkalinity on SPF tests, the spontaneous alkalinity value (330 mg L^{-1}) was
191 adjusted to 75, 150 and 600 mg L^{-1}, respectively. Specifically, 2.8 mL of H_2SO_4 concentrated solution
192 (95%) were added to 30 L wastewater to get a final alkalinity of 75 mg L^{-1} (pH = 6.8) and 1.5 mL in
193 30 L for a final alkalinity of 150 mg L^{-1} (pH = 7.0). The final samples were purged overnight by an
194 air bubbler to remove CO_2 formed following the acidification. To increase the alkalinity to 600 mg
195 L^{-1}, $NaHCO_3$ (99%) was added to the sample (final pH = 7.5).
196

197 ***2.4 Analytical measurements***

198 PCs concentration was measured by Ultra-Performance Liquid Chromatography (Ultimate^{TM} 3000
199 Basic Automated System, ThermoScientific) equipped with an UV-DAD detector, column thermostat
200 and an automated sample injector (loop volume: 100 µL). PCs separation was achieved on a reversed-
201 phase Luna C18 column (150x4.6 mm, 5 µm, 100 Å), by using gradient elution with 0.1% formic
202 acid in water (v/v, eluent A) and 0.1% formic acid in acetonitrile (v/v, eluent B) at a flow rate of 1
203 mL min^{-1}. The gradients were as follows: 0-10 min from 2% to 85% B; 10-12 min to 85% B; 12-13
204 min from 85% to 2% B (initial conditions); 2 min to 2% B (reconditioning). The column temperature
205 was set at 40°C. The injection volume was 10 µL. Wavelength of maximum absorbance for SMX,
206 CBZ and TMP was 270 nm. Retention times for TMP, SMX and CBZ were 6.54 min, 8.67 min and
207 9.43 min, respectively.

208 Aqueous samples for the analytic measurements (10 mL) were collected using a 15 mL plastic syringe
209 (10 mL) at fixed time intervals, filtered through 0.45 μm PTFE filter (Merck Millipore), if PCs
210 determination was needed; for bacteria enumeration, this last operation was ruled out.
211 Spectrophotometric determination of iron and H_2O_2 concentration was performed by a UV/Vis
212 Lambda-25 spectrophotometer (Perkin Elmer). H_2O_2 concentration was determined according to DIN
213 38 402 H15 method: measurement at 410 nm wavelength of the solution obtained adding 0.3 mL of
214 titanium (IV) oxysulfate to 3 mL sample previously filtered (0.45 μm PTFE filter) was carried out.
215 Dissolved Fe concentration was measured according to ISO 6332:1988 method, by analyzing the
216 absorbance at 510 nm wavelength of the solution obtained by adding 1,10-phenantroline to 10 mL of
217 previously filtered (0.45 μm PTFE filter) sample and subsequently acidified.
218 Chemical Oxigen Demand (COD), Alkalinity, Total Nitrogen, Nitrites and Nitrates concentrations
219 were determined spectrophotometrically using the appropriate test kit (HACH). BOD_5 was measured
220 by manometric method pouring freshly collected secondary wastewater (432 mL) in Oxitop®
221 respirometer bottles (Xylem, USA). pH was measured by a pH meter (HANNA Edge®), the
222 instrument being calibrated before its use. Turbidity measurements were obtained by a HACH 2100N
223 Turbidimeter.
224

225 ***2.5 Microbiological analysis***

226 Samples (60 mL) taken during SPF and sunlight/H_2O_2 experiments were mixed with 1.2 mL of bovine
227 liver catalase solution (0.2 g L^{-1} stock solution in deionized water) to quench H_2O_2 residual
228 concentration before microbial analysis. Subsequently, enumeration of bacteria was performed by
229 standard plate counting method (Double Agar Layer, lysis plaque method for somatic coliphages)
230 and filtration membrane method (all the experiments were carried out in triplicate) using the
231 following media: Triptone Bile X-Glucoronide and Triptone Soy Agar for *E. coli*; m-CP Agar (mixed
232 with polymyxin B sulfate, D-cycloserin and indoxyl-β-D glucoside) for *C. perfringens*; Triptone Agar

(mixed with nalidixic acid for somatic coliphages; with ampicillin sodium salt and streptomycin for F-plus coliphages) and inseminated with a solution of *E. coli* ATCC 13706 strain or *E. coli* ATCC 15597 for coliphages (dual layer agar technique). Plates were incubated at 37°C for 24 h and at 44°C for 24 h (in anaerobic jar) for *E. coli* and *C. perfringens*, respectively. For coliphages, an aliquot of a 18 h cultured host selected bacterial strain was mixed with overlay agar (pre-heated at 45°C).

3. Results and discussion

3.1 Effect of operating conditions on SPF performances

The operating conditions of the SPF process with the two catalysts under study (Fe:EDDS and Fe:IDS) were optimized by evaluating: (i) the effect of wastewater alkalinity on PCs removal and *E. coli* inactivation; (ii) the effect of Fe concentration and Fe:L ratio on PCs removal. In all trials the initial H_2O_2 dosage was set at 50 mg L^{-1}.

3.1.1 Effect of wastewater alkalinity on PCs removal and E. coli inactivation

Although the negative effect of alkalinity on the AOPs efficiency is well known, as carbonates act as hydroxyl radical ($HO^{\bullet}$) scavengers (Pignatello et al., 2006; Papoutsakis et al., 2015) and are able to lead to the break-up of iron chelates (Miralles-Cuevas et al., 2014), in this work such effect was evaluated systematically to verify to which extent alkalinity comparatively affects the two complexes. The higher the alkalinity, the lower SPF process efficiency in terms of PCs degradation (Figure 1a and 1b). Interestingly, the effect of the alkalinity is more pronounced for EDDS than IDS. As the alkalinity increased from 75 mg L^{-1} to 150 mg L^{-1}, a drastic decrease in the mean value of PCs removal efficiency (from 78% to 50%) was observed for the Fe:EDDS supported process (Figure 1a), while a lower decrease was observed for Fe:IDS (from 58% to 38%) (Figure 1b). Taking into account that radical species attack in a non-selective manner all the organic compounds (Deng and Zhao, 2015), the most probable explanation for the differences detected between these two complexes is given by

the inferior capability of generating radical species by Fe:IDS in presence of sunlight and H_2O_2. The higher capability of generating $HO^{\bullet}$ by Fe:EDDS was partially thwarted by the preferential attack of the produced radicals to the more abundant ligand and carbonates rather than PCs (only 0.2 mg L^{-1}). Indeed, the reaction of $HO^{\bullet}$ with carbonates (CO_3^{2-}/HCO_3^-) leads to the formation of carbonate radicals ($HCO_3^{\bullet}/CO_3^{\bullet-}$) that are 3 times less reactive than $HO^{\bullet}$ worsening the side effects of radical scavenging phenomenon (Ji et al., 2015; Lofrano et al., 2021). In parallel, the effect of alkalinity on wastewater disinfection was evaluated using *E. coli* as target bacteria, being among the most used indicators for wastewater disinfection and reuse and recently included in the first European regulation on wastewater reuse for agricultural applications (EU 2020/741). Consistently with the results for PCs removal, the optimal operative conditions were proved to be 75 mg L^{-1} in terms of alkalinity. (Figure S2).

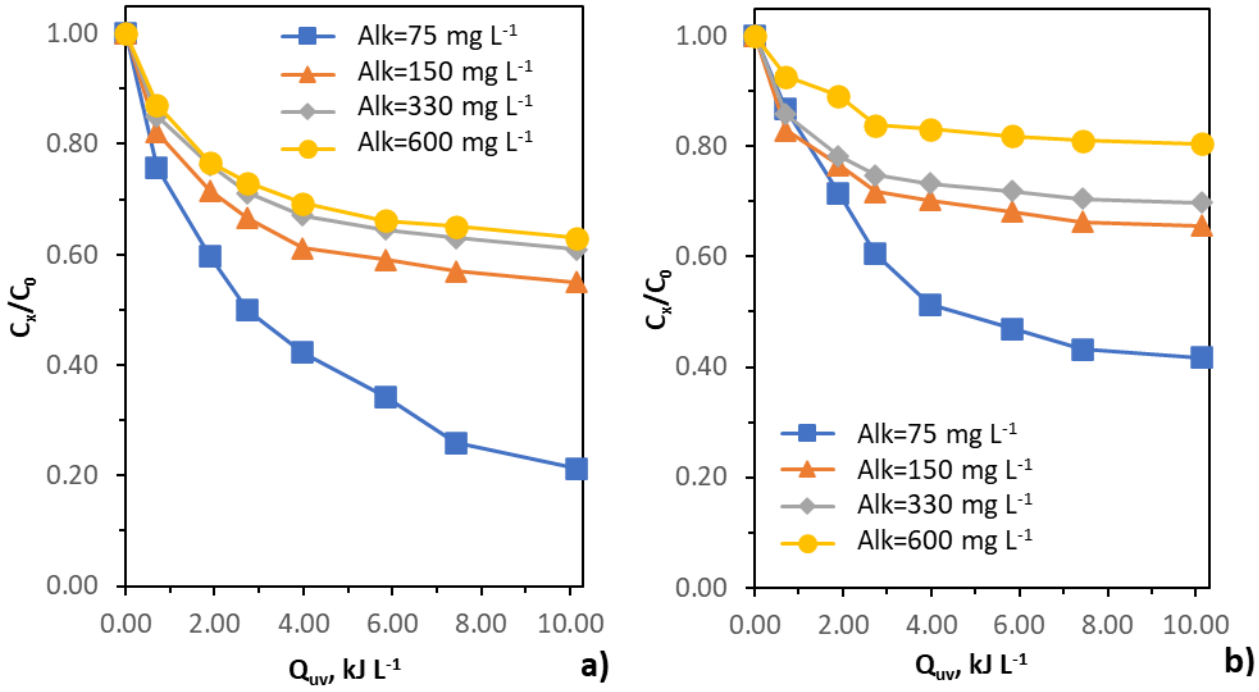


Figure 1. Effect of wastewater alkalinity (from 75 mg L^{-1} to 600 mg L^{-1}) on PCs degradation by SPF process with Fe:EDDS (a) and Fe:IDS (b). Total time=180 min, H_2O_2=1.47 mM, Fe:L=0.1 mM:0.1 mM.

3.1.2 Effect of Fe concentration and Fe:L ratio on PCs removal

Once the optimal alkalinity condition for PCs removal was identified, the subsequent experiments, aimed at optimizing the catalyst dosage (Fe concentration and Fe:L ratio), were carried out under the same alkalinity conditions (75 mg L^{-1}). Indeed, while H_2O_2 dose in SPF like process for tertiary treatment of urban wastewater is well known from the literature (De La Cruz et al., 2013; Fiorentino et al., 2019), iron initial dose and Fe:L ratio can change in a narrow range, depending on wastewater characteristics, and significantly affecting process efficiency. Accordingly, three iron doses and two Fe:L ratios were investigated. The best operative condition resulted to be 0.1 mM Fe, 0.1 mM for both chelating agents (Table 2). SPF with Fe:EDDS (0.1 mM, molar ratio Fe:EDDS = 1:1, H_2O_2 = 50 mg L^{-1}) was effective in the degradation of the target PCs, in particular in the early 60 min ($Q_{uv=}$ 4.14 kJ L^{-1}) of the experiment where the average removal was as high as 61% (Figure S1). Consistently with these results, the H_2O_2 consumption rate was fast (Figure S1) and relevant in the early 60 min (77%). SMX showed to be the most degradable contaminant (82%), followed by CBZ (77%) and TMP (76%) (Table 2). Subsequently, the removal rate slowed down, and from 120 min (Q_{uv}= 7.4 kJ L^{-1}) until the end of the test, PCs residual concentrations did not show a remarkable decrease. These results are consistent with previous works available in literature under comparable operating conditions (Maniakova et al., 2022a).

Noteworthy, an increase in Fe concentration (from 0.1 mM to 0.2 mM) or in the Fe:L ratio (from 1:1 to 1:1.5) did not positively affect the process, regardless of the ligand exploited. EDDS can be an easy target for $HO^{\bullet}$ (Wu et al., 2014), therefore the decreased efficiency of the process as the ligand dose was increased can be due to a massive consumption of radicals, leading to unsatisfactory results in terms of PCs removal. A similar result was also observed for IDS. Nevertheless, when Fe:IDS was investigated (0.1 mM, molar ratio Fe:IDS = 1:1, H_2O_2 = 50 mg L^{-1}), a lower efficiency in the degradation of the target PCs was observed. Namely, SMX, CBZ and TMP were reduced up to 69%, 51% and 55%, respectively, after 180 min (12.8 kJ L^{-1}) (Table 2). Noteworthy, the concentration of

295 dissolved iron decreased faster in Fe:EDDS based SPF experiment. Indeed, iron almost totally
296 precipitated (3 mg L^{-1}, Figure S1) after 60 min (Q_{uv}= 4.2 kJ L^{-1}), compared to Fe:IDS based SPF
297 experiment (2.4 mg L^{-1}, Figure S1).
298 Iron precipitation resulted in a higher increase in wastewater turbidity for the Fe:EDDS complex
299 (from 0.5 to 3.0 NTU after 60 min) compared to the Fe: IDS complex (from 0.5 to 1.1 NTU). Possibly
300 the slower activity of the Fe:IDS complex resulted in a lower ROS production rate with a consequent
301 reduced degradation of the ligand which finally keeps the iron in the dissolved phase longer. This
302 ligand, like EDDS, can play a competing role for oxidating species at the same time. Lastly, reducing
303 Fe concentration from 0.1 mM to 0.05 mM led to poor outcomes, as a consequence of the reduced
304 production of radicals involved in the oxidation of the target PCs.
305
306 **Table 2**. Effect of Fe concentration and Fe:L ratio on the PCs removal efficiency. H_2O_2=1.47 mM, alkalinity=75 mg L^{-1},
307 Q_{uv}=12.9 kJ L^{-1} (180 min).

Catalyst	Fe^{3+}, mM	Fe:L ratio	TMP removed, %	CBZ removed, %	SMX removed, %
Fe:EDDS	0.05	1:1	38.4	19.5	40.7
	0.1	1:1	75.5	76.5	82
	0.1	1:1.5	48.1	43.7	55
	0.2	1:1	50.3	56.6	67.2
Fe:IDS	0.05	1:1	15.7	20.2	20.6
	0.1	1:1	55.2	50.6	69.4
	0.1	1:1.5	39.8	37.5	43.4
	0.2	1:1	39.6	37.6	52.1

308

309 ***3.2 Effect of SPF on pathogens inactivation: comparison with sunlight/H_2O_2 process***
310 After the working conditions of SPF were optimized in terms of PCs removal (Fe:L = 0.1:0.1 mM),
311 the effect of these conditions on *E. coli*, *C. perfringens* and coliphages (somatic and F-plus)
312 inactivation was investigated. The target pathogens for disinfection's performance evaluation were
313 selected according to the new EU regulation on wastewater reuse (EU 2020/741). The SPF process

was also compared with the sunlight/H_2O_2 process as a benchmark disinfection process. While sunlight/H_2O_2 process poorly affects the PCs decomposition because of the poor generation of $HO^{\bullet}$ under natural solar radiation (Giannakis et al., 2016a; Moreira et al., 2018; Berruti et al., 2022; Michael et al., 2020), it is an effective disinfection process (Ferro et al., 2015; García-Gil et al., 2022). SPF with the two catalysts (Fe:IDS and Fe:EDDS) showed opposite behaviors for pathogens inactivation with respect to what observed for PCs removal (Figure 2; Table S1-S3). Quite surprisingly, Fe:EDDS (Figure 2a) resulted in reduced disinfection efficiency compared to Fe:IDS counterpart (Figure 2b). Noteworthy, SPF with Fe:IDS allowed to achieve more than four log units inactivation as well as to decrease residual *E. coli* concentration below the limit set by EU regulation for wastewater reuse for class A water (10 CFU 100 mL^{-1}) in 180 min treatment (Q_{uv}=15.9 kJ L^{-1}). On the opposite, SPF with Fe:EDDS resulted in a comparable poor efficiency with nearly two log units inactivation, but consistent with previous results (Maniakova et al., 2021b).

The only apparent contradictory outcomes for Fe:IDS and Fe:EDDS based SPF processes in the disinfection experiments, if compared with those obtained for the PCs removal, actually can be explained as follows. While PCs degradation is a process almost exclusively dependent on the amount of produced radical species, a different scenario takes place in the disinfection process. Indeed, it is widely reported in the scientific literature how an important role in bacteria inactivation is played not only by the radical species produced through the external SPF process, which damage the cell wall, but even by the intracellular photo-Fenton mechanism triggered by the solar radiation (Fisher et al., 2014; Giannakis et al., 2016a; Giannakis et al., 2016b; Giannakis et al., 2018; García-Gil et al., 2022; Giannakis et al., 2022). The internal photo-Fenton reaction takes place thanks to H_2O_2 permeating the cell membrane reacting with the bioavailable and externally added iron ions. Indeed, in Fe:EDDS based SPF, the significantly higher consumption of H_2O_2 and the faster iron precipitation (Figure S3), due to the degradation of the chelating agent, resulted in a higher turbidity (3 NTU) compared to Fe:IDS tests (1.1 NTU). Presumably, the higher turbidity in Fe:EDDS tests acted as a screen for

sunlight, reducing its penetration inside bacteria cells and consequently resulting in a less effective internal photo-Fenton mechanism.

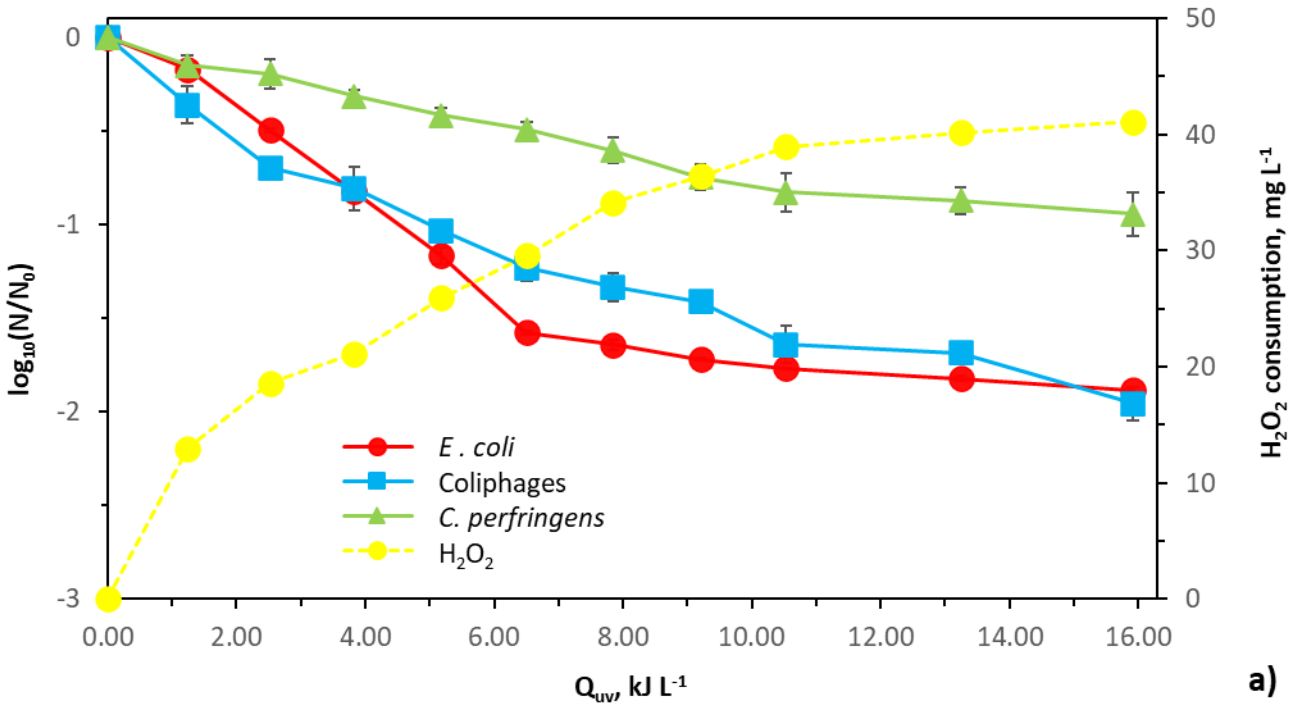
Interestingly, SPF with Fe:IDS was also more effective than sunlight/H_2O_2 process in *E. coli* inactivation (Figure 2b and 2c, 3.3 log units inactivation). Consistently, sunlight/H_2O_2 process performed better than Fe:EDDS based SPF process (Figure 2a and 2c), confirming previous literature results (Maniakova et al., 2021b).

The inactivation efficiency of sunlight/H_2O_2 is mainly due to the aforementioned intracellular photo-Fenton process, while in SPF this mechanism is coupled to the extracellular production of HO• and other radical species that strongly contribute to membrane damaging, favoring the permeation of H_2O_2 and Fe ions inside the cells (Rommozzi et al., 2020). For this reason, the lower reactivity of Fe:IDS was found to be an advantage for the disinfection process. The lower production of HO• results in a reduced degradation of the chelating agent with a lower turbidity of the aqueous matrix which, in addition to the residual concentration of H_2O_2 (Figure 2b), make the internal photo-Fenton reaction more effective. In summary, the three processes were found to be effective in *E. coli* inactivation in the order: Fe:IDS based SPF > sunlight/H_2O_2 > Fe:EDDS based SPF.

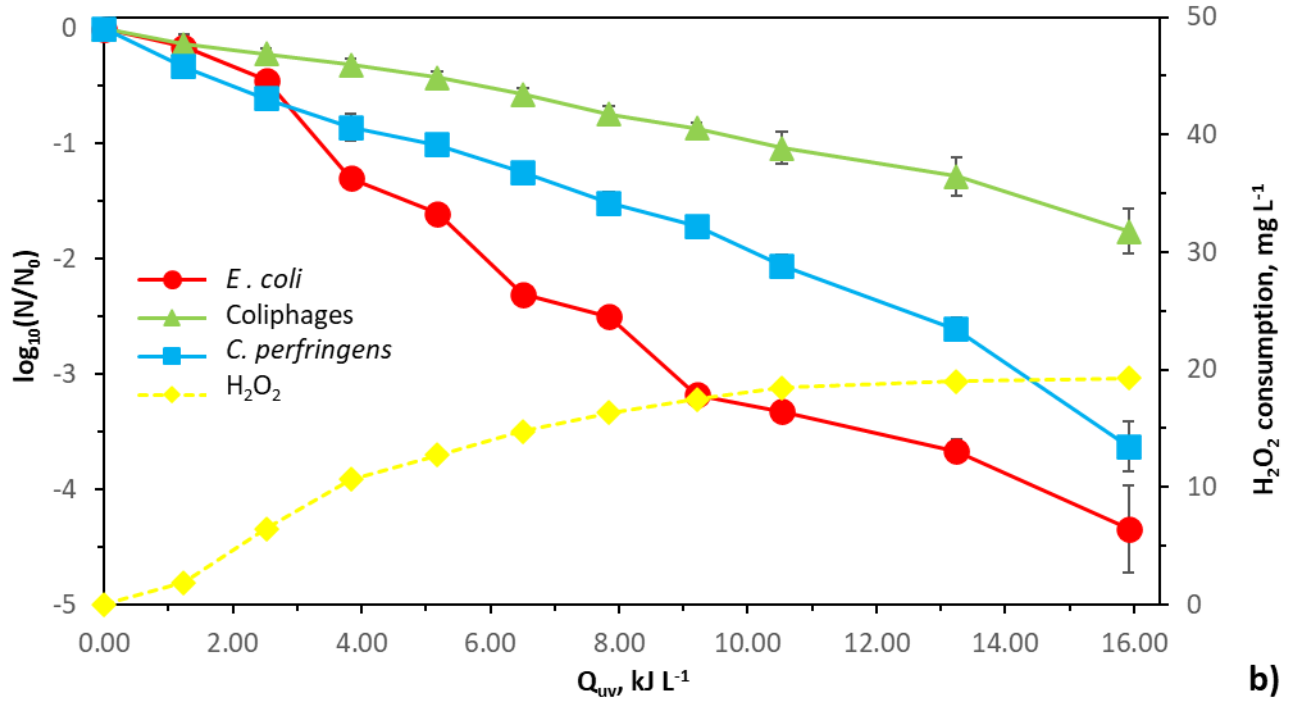
At the same time, the effect of solar driven AOPs on the inactivation of other pathogens relevant for the new EU regulation on wastewater reuse, namely *C. perfringens* and coliphages (somatic and F-plus), was investigated. The order of inactivation of the target pathogens by Fe:IDS based SPF and sunlight/H_2O_2 process was *E. coli* (under the DL of 1 CFU 100 mL^{-1}) > coliphages > *C. perfringens*, while for Fe:EDDS the following order was observed: coliphages>*E. coli*>*C. perfringens* (Figure 2a, 3b and 3c). According to the scientific literature, differences occur between bacterial and viral disinfection for SPF processes, with internal photo-Fenton playing a key-role in the disinfection for bacteria and external ROS induced damage being the main pathway for inactivation of viruses. Moreover, bacteriophages are more susceptible than bacteria to oxidation damage (Carratalà et al., 2016; Giannakis et al., 2018). Again, Fe:IDS based SPF proved to be more effective than sunlight/H_2O_2 and Fe:EDDS based SPF in coliphages inactivation. The high susceptibility of

coliphages to oxidative damage explains the better performance of the Fe:IDS based SPF compared to sunlight/H_2O_2. At the same time, the result seems contradictory considering that Fe:EDDS is able to produce more radical species. A possible explanation can be found in the residual concentration of H_2O_2 always higher in the SPF with Fe:IDS than in the SPF with Fe:EDDS. Furthermore, a strong correlation among host bacteria abundance and bacteriophages resistance exists, according to the literature; in particular, the more damaged the *E. coli*, the less the probability for coliphages to continue their life-cycle (Voumard et al., 2019). Therefore, coliphages inactivation is directly correlated to other bacteria inactivation. Moreover, the lesser precipitation of iron showed by Fe:IDS based SPF (Figure S3) allows sunlight to play an important role in the disinfection. The important role played by sunlight for viruses' disinfection has been clearly described in literature (Silverman et al., 2019), therefore an iron complex both able to generate radical species, and stable enough to delay iron precipitation and turbidity increasing, is desirable for bacteriophages inactivation.

Lastly, in all the processes *C. perfringens* resulted to be the most resistant pathogen. *C. perfringens* is a gram-positive bacterium and, according to the scientific literature gram-negative and gram-positive bacteria are characterized by different susceptibility to AOPs (Van Griecken et al., 2010; Rodríguez-Chueca et al., 2013). Indeed, gram-negative (e.g., *E. coli*) ones bear an outer lipopolysaccharidic membrane, which surrounds a thin peptidoglycan layer and an inner cytoplasmic membrane (Silhavy et al., 2010). Instead, the gram-positive bacteria own a thicker layer of peptidoglycans (Kloos et al., 1999). This difference in membrane composition can affect the cell permeability (Frallicciardi et al., 2022), making the microorganism membrane more or less likely to be penetrated by chemical compounds and/or hardly to resist to ROS attacks. In addition, a serious threat posed by *C. perfringens* consists in being a spore-forming bacterium, able to generate resistant forms (endospores) to protect themselves from adverse chemical-physical conditions as the presence of ROS (Granger et al., 2011). In fact, spores are particularly resistant to SPF process (Kokkinos et al., 2021).


log10(N/N0)
H2O2 consumption, mg L-1
Quv, kJ L-1
E . coli
Coliphages
C. perfringens
H2O2
a)

390


log10(N/N0)
H2O2 consumption, mg L-1
Quv, kJ L-1
E . coli
Coliphages
C. perfringens
H2O2
b)

391

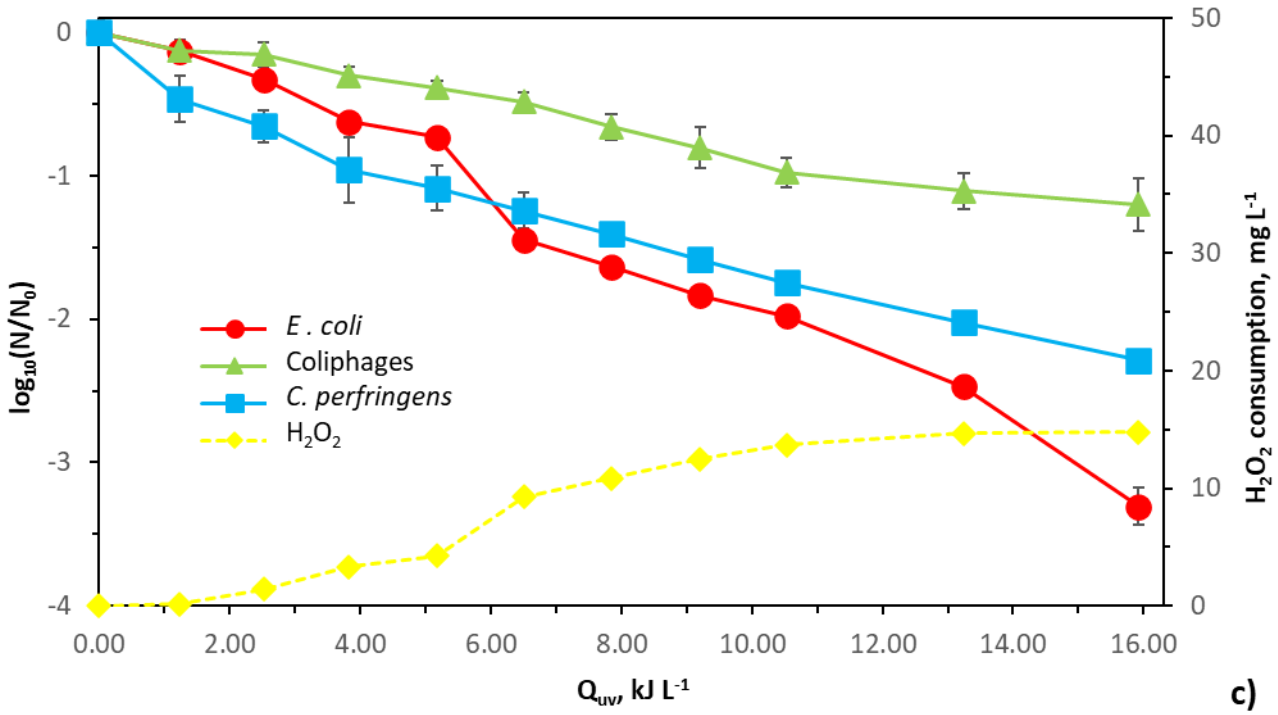


Figure 2. Inactivation of *E. coli*, coliphages and *C. perfringens* by Fe:EDDS based SPF (a), Fe:IDS based SPF (b) and sunlight/H_2O_2 process (c). Total time=180 min; H_2O_2=1.47 mM, Fe:L=0.1 mM:0.1 mM.

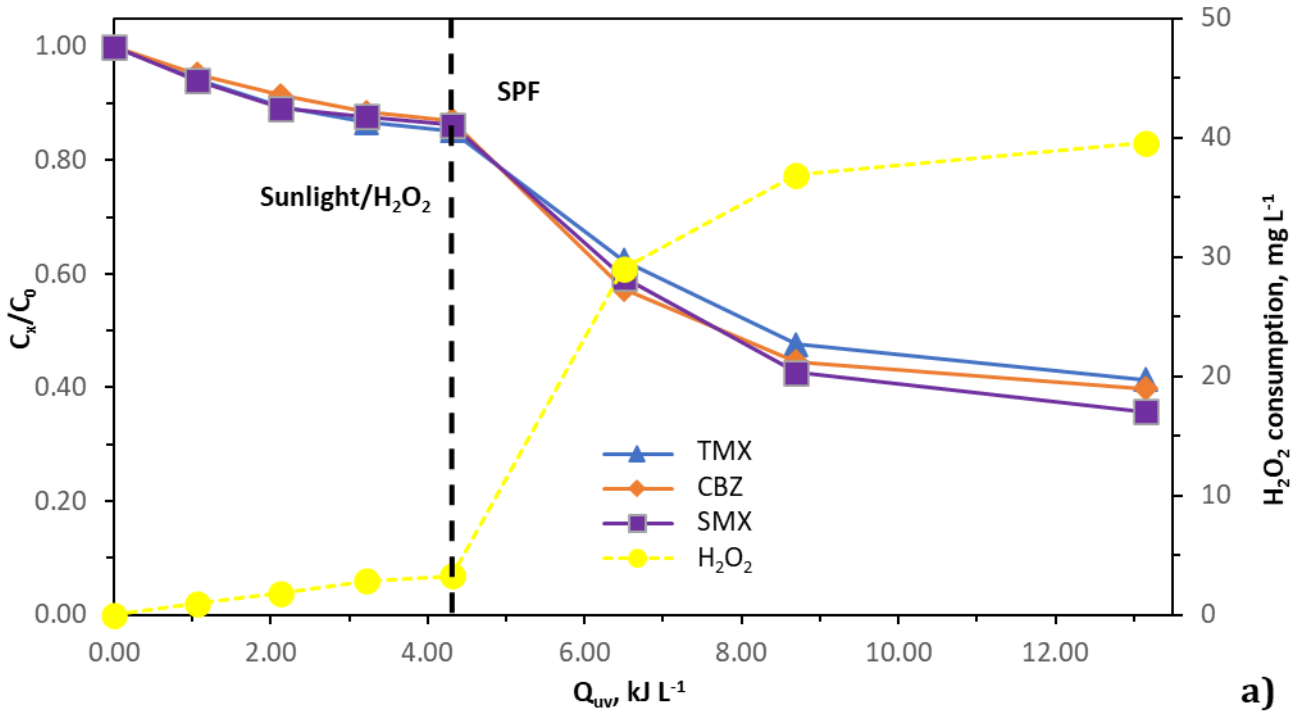
3.3 Sequential treatment: Sunlight/H_2O_2-SPF

Sunlight/H_2O_2 resulted more effective as disinfection process compared to Fe:EDDS based SPF (Figure 2a and 2c). On the other hand, it is quite ineffective in the PCs degradation, being not able to generate a sufficient amount of radicals unlike of SPF (Michael et al., 2020; Maniakova et al., 2021b). Therefore, Maniakova et al. investigated for the first time the sequential combination of sunlight/H_2O_2 and SPF with Fe:EDDS obtaining simultaneous PCs removal and disinfection in real urban wastewater (Maniakova et al., 2022b). In our study, Fe:EDDS and Fe:IDS were also compared on their effects on PCs an pathogens in the sequential sunlight/H_2O_2-SPF treatment for the first time. For all the sequential treatment experiments, the total time remained unchanged (180 min), while the SPF phase lasted 120 min (about 8 kJ L^{-1}) to achieve a satisfactory PCs removal (limiting factor in the combination of sunlight/H_2O_2 and SPF) according to what was obtained from the previous experiments (Figure 1; Figure S1).

3.3.1 PCs removal

As PCs removal is of concern, sequential treatment with Fe:IDS was less efficient than the combined process with Fe:EDDS (Figure 3a and 3b), consistently with the previous results on the application of SPF as stand-alone process (Figure S1). In details, the average PCs removal was around 61% for the sequential sunlight/H_2O_2-Fe:EDDS based SPF process, while for the Fe:IDS based SPF only 47% was reached. Obviously, as in the early 60 min (Q_{uv}= 4.2 kJ L^{-1}) the two conditions tested coincide (sunlight/H_2O_2), sequential treatments differ only for the chelating agent used in the last 120 min (Q_{uv} ≈ 9 kJ L^{-1}) of SPF. Sunlight/H_2O_2 had a limited effect on PCs removal (about 14%) according to scientific literature (Michael et al., 2020; Maniakova et al., 2021b). Again, sequential treatment with Fe:EDDS based SPF resulted more performant then sequential treatment with Fe:IDS based SPF for the average PCs removal (61% vs 47%) (Figure 3). Recently, a study on the simultaneous removal of PCs (five compounds at a concentration of 0.1 mg L^{-1}) and inactivation of pathogens (*E. coli* and *Salmonella spp*.) by the sequential treatment sunlight/H_2O_2-Fe:EDDS based SPF reported an average PCs removal of 60% (Maniakova et al., 2022b).While confirming the best performance of Fe:EDDS in PCs removal, the sequential treatment was less effective than SPF stand-alone processes for both catalysts (Figure 3, Figure S1). Since in the sequential treatment sufficient energy was accumulated during the SPF phase compared to the previous results (about 9 kJ L^{-1}), the explanation for this outcome may be due to the lower H_2O_2 concentration at the beginning of the SPF due to its consumption during the preceding sunlight/H_2O_2 phase (Figure 3). This decreases the initial H_2O_2/Fe ratio which can negatively affect the production of $HO^{\bullet}$ and the PCs degradation, especially in processes involving organic chelating agents (Lumbaque et al., 2019; Lin and Lin, 2021). Furthermore, not only H_2O_2 can be an influencing factor, but also transformation products generated by the sunlight/H_2O_2 phase can contribute to the matrix effect. Indeed, sunlight/H_2O_2 is not able to mineralize the target contaminants which finally generate transformation products (Rizzo et al., 2019;

Michael et al. 2020). This implies that the PCs degraded during the sunlight/H_2O_2 phase are transformed in byproducts that can possibly affect the SPF reaction.



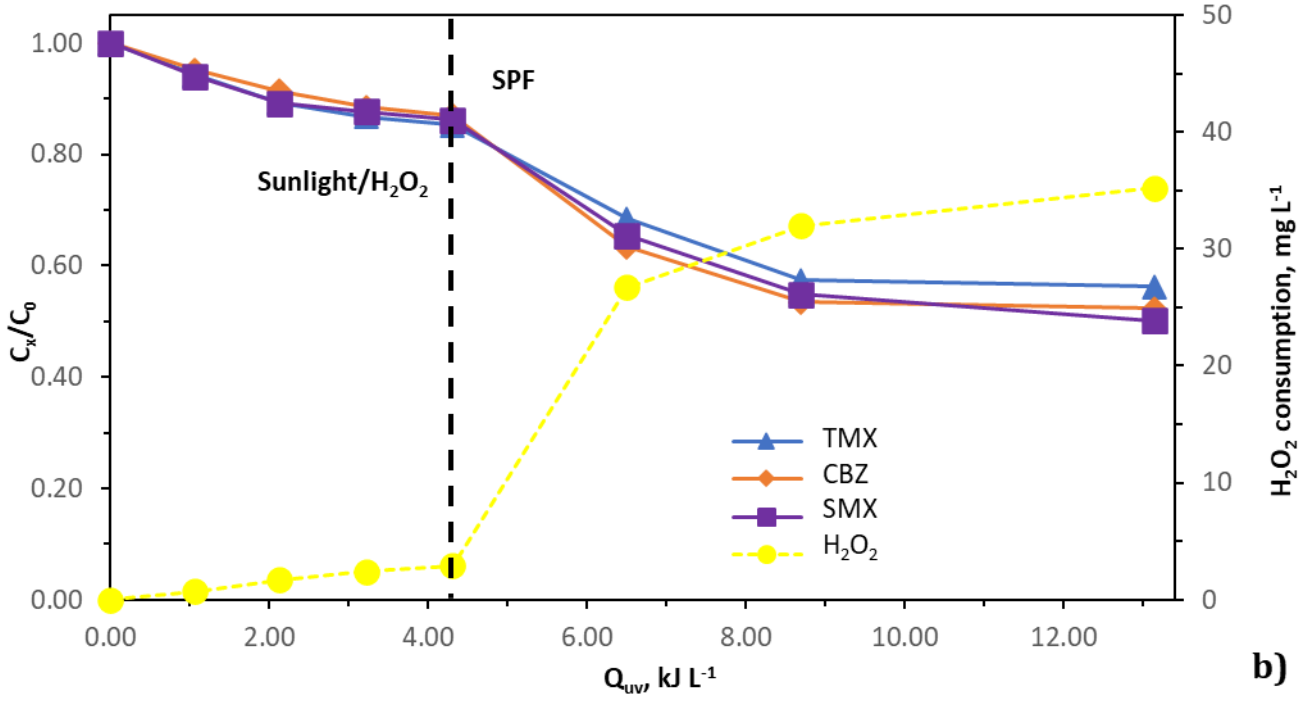
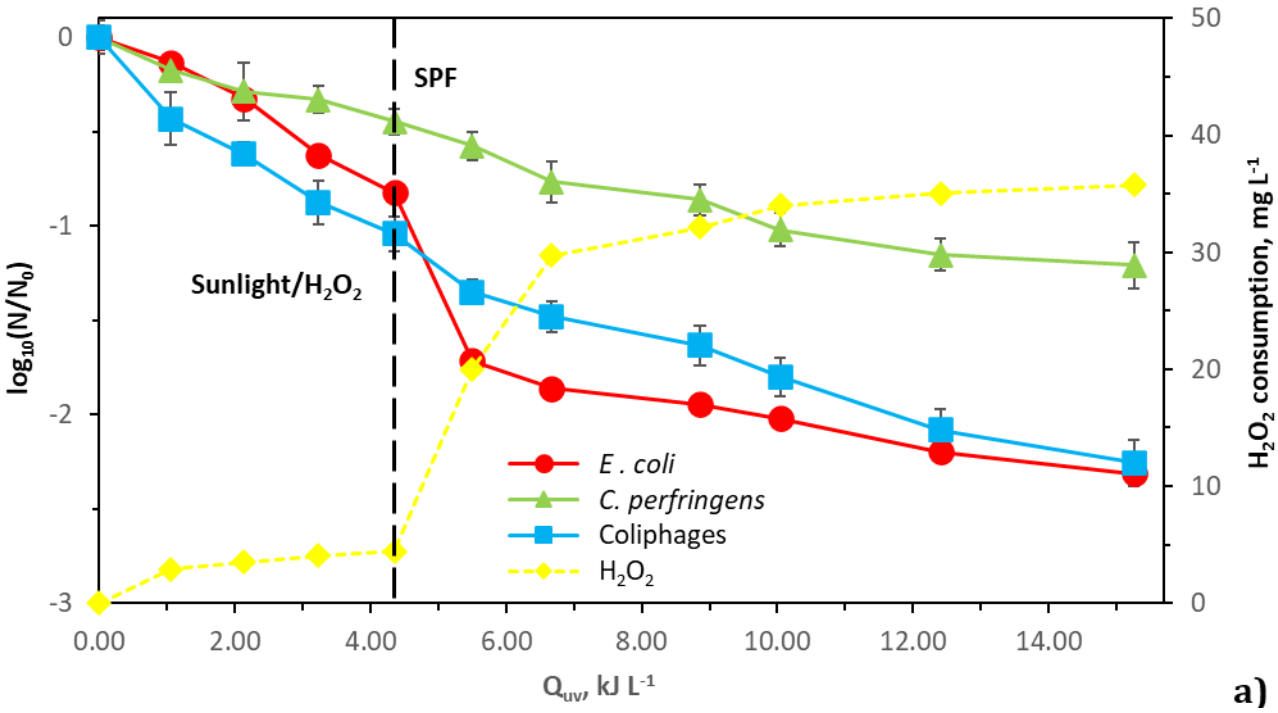


Figure 3. PCs removal by sequential treatment sunlight/H_2O_2 – Fe:EDDS (a) and sunlight/H_2O_2 – Fe:IDS (b). Total time=180 min; H_2O_2=1.47 mM; Fe:L=0.1 mM:0.1 mM.

3.3.2 Pathogens inactivation

The effect on pathogens inactivation was also evaluated for sequential treatment sunlight/H_2O_2-SPF (Figure 4; Table S4-S5). In sequential treatment, the Fe:IDS complex led to higher disinfection efficiencies than the Fe:EDDS complex for all pathogens (3.4 vs 2.3 log reduction for *E. coli*, 2.6 vs 2.3 log reduction for coliphages, 1.5 vs 1.2 log reduction for *C. perfringens*, respectively). For both chelating agents, contributions to the disinfection can be considered to stem from two separate steps: firstly, sunlight/H_2O_2, then Fe:L based SPF. In the common first step, as previously discussed, disinfection is driven by an internal photo-Fenton process. On the opposite, in the second step, two pathways are possible. Consistently with the discussion about the effect of SPF on pathogens inactivation, after adding Fe:IDS (Figure 4b), disinfection is driven by intracellular photo-Fenton conjugated with extracellular photo-Fenton. With Fe:EDDS (Figure 4a), H_2O_2 and Fe ions are quickly consumed outside the cells, the turbidity increases faster, thus making the intracellular photo-Fenton mechanism less relevant compared to Fe:IDS. The progressive and almost total consumption of H_2O_2 in the Fe:EDDS based SPF phase (Figure 4a) led to a reduction in the pathogens inactivation rate. In contrast, in sequential treatment with Fe:IDS based SPF, the pathogen inactivation rate continued to increase, consistently with the high H_2O_2 availability until the end of treatment. Moreover, the sequential treatment with Fe:IDS based SPF (Figure 4b) showed a lower efficiency than its corresponding SPF (Figure 2b) as a stand-alone process (3.4 vs 4.3 log reduction for *E. coli*, 2.6 vs 3.6 log reduction for coliphages, 1.5 vs 1.8 log reduction for *C. perfringens*). This worsening confirms the hypothesis on the disinfection mechanisms involved in the Fe:IDS based SPF. Indeed, by coupling the sunlight/H_2O_2 process to SPF, the time in which both mechanisms (internal and external photo-Fenton) coexist is reduced (from 180 min to 120 min). On the other hand, and according to the only previous work reporting this comparison (Maniakova et al., 2021b), the sequential treatment with Fe:EDDS based SPF (Figure 4a) showed a higher efficiency then its corresponding SPF (Figure 2a) as a stand-alone process (2.3 vs 1.9 log reduction for *E. coli*; 2.3 vs 2 log reduction for coliphages,

1.2 vs 0.9 log reduction for *C. perfringens*). The increased pathogen inactivation in this case may be due to cell wall weakening caused by the internal photo-Fenton during the sunlight/H_2O_2 phase. This had a beneficial effect especially on *E. coli* inactivation during the SPF. Indeed, *E. coli* is the only one of the three pathogens to undergo an increase in disinfection rate after SPF started (Figure 4a). This result can be explained through the higher susceptibility of this bacterium in being permeated and damaged by H_2O_2 due to the weaker cell wall compared to *C. perfringens* (Van Griecken et al., 2010; Rodríguez-Chueca et al., 2013; Frallicciardi et al., 2022).



a)

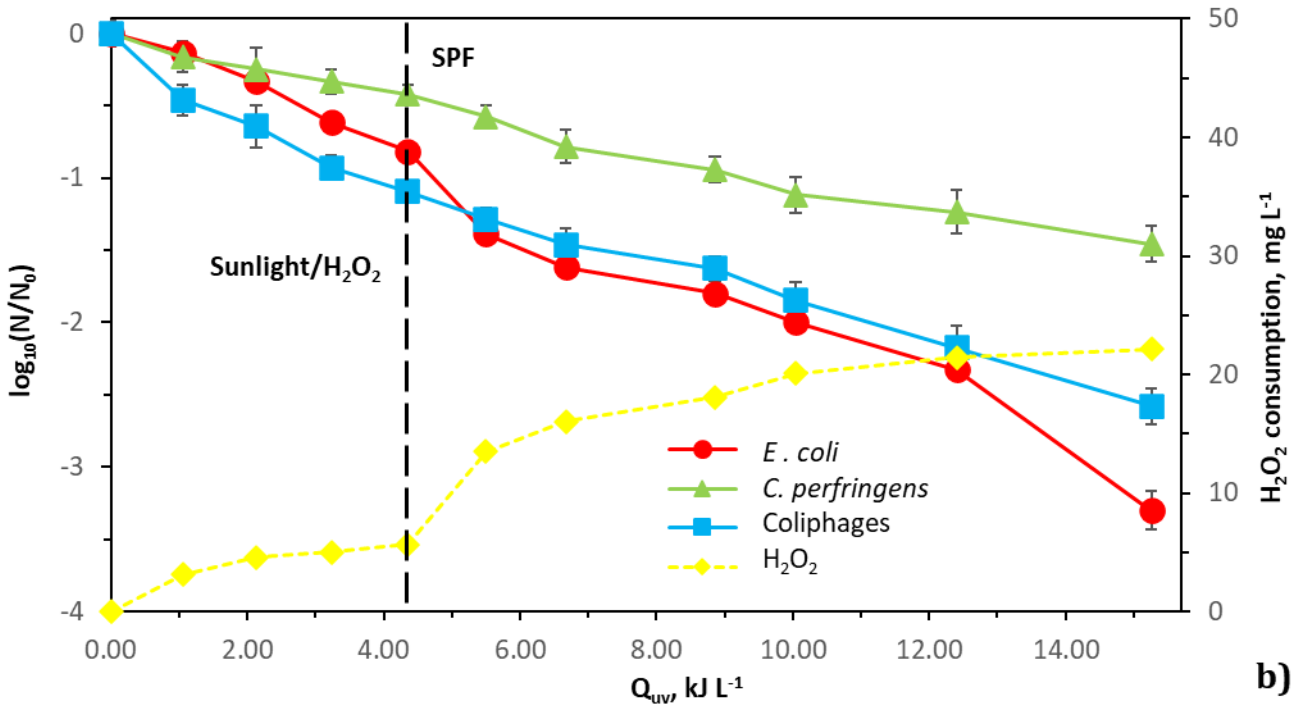


Figure 4. Inactivation of *E. coli*, coliphages and *C. perfringens* by sequential treatment: sunlight/H_2O_2 – SPF with Fe:EDDS (a) and Fe:IDS (b). Total time=180 min; H_2O_2=1.47 mM; Fe:L=0.1 mM:0.1 mM.

Conclusions

In this study, the Fe:IDS complex as a catalyst for SPF process at natural pH was investigated for the first time as a tertiary treatment for wastewater reuse in agriculture applications. The Fe:IDS based SPF was compared with the well-known Fe:EDDS based SPF and then with the sequential treatment configuration (sunlight/H_2O_2-SPF) in terms of pathogens (*E. coli*; *C. perfringens*; coliphages) inactivation and PCs (SMX, CBZ, TMP) removal from real secondary wastewater. Both Fe:L based SPF treatments were found to be effective in the simultaneous disinfection and PCs removal, but Fe:IDS based SPF resulted the best option for pathogens inactivation, allowing to meet the most stringent limit (Class A = 10 CFU/100 ml) for *E. coli* set by EU regulation for wastewater reuse (2020/441). Moreover, the process also affected *C. perfringens* and coliphages that are relevant for the wastewater treatment plant validation for wastewater reuse. On the other hand, Fe:IDS based SPF was found to be less effective for PCs degradation compared to Fe:EDDS based SPF. The main explanation for the differences between the two catalysts is that the lower reactivity of Fe:IDS results

in a reduced consumption of Fenton's reagents favouring both the intracellular and extracellular photo-Fenton mechanisms for disinfection but lowering its PCs degradation potential. Noteworthy, while for Fe:EDDS the sequential treatment increased the disinfection efficiency, for the Fe:IDS the sunlight/H_2O_2 step was deleterious in terms of performances.

Considering its good all-round performance, but also IDS higher biodegradability, Fe:IDS can be an attractive alternative option to Fe:EDDS in SPF process for the tertiary treatment of urban wastewater in small WTPs, especially when more stringent limits on pathogens should be meet (as in the case of the new EU regulation on wastewater reuse) and residual PCs in the effluent is consistent with the corresponding treatment target.

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