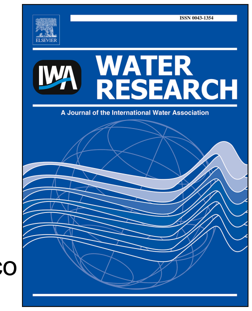
Accepted Manuscript



Disinfection of urban wastewater by a new photo-Fenton like process using Cu-iminodisuccinic acid complex as catalyst at neutral pH

Antonino Fiorentino, Raffaele Cucciniello, Andrea Di Cesare, Diego Fontaneto, Prisco Prete, Luigi Rizzo, Gianluca Corno, Antonio Proto

PII: S0043-1354(18)30644-4

DOI: 10.1016/j.watres.2018.08.024

Reference: WR 14000

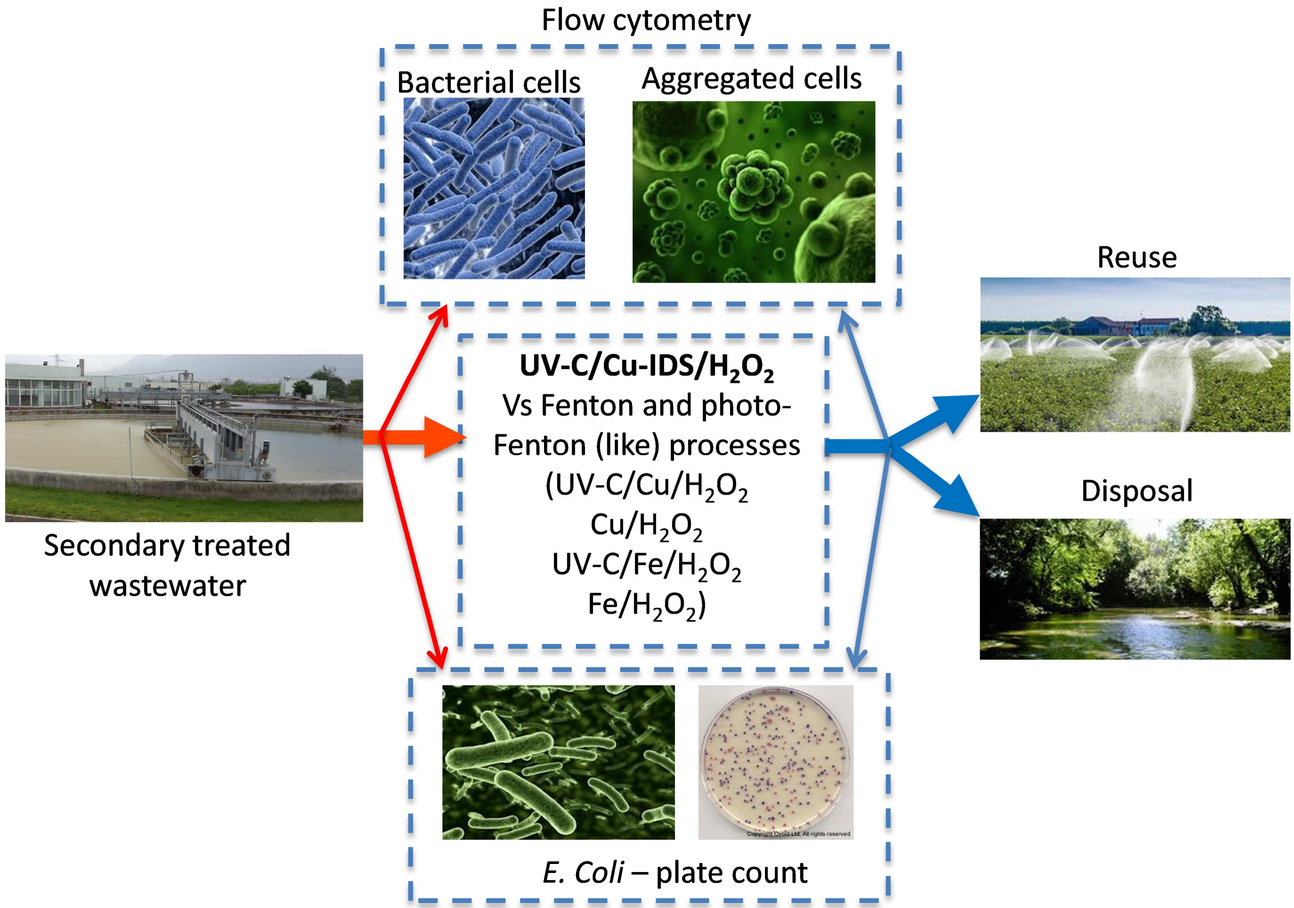
To appear in: *Water Research*

Received Date: 11 May 2018

Revised Date: 24 July 2018

Accepted Date: 9 August 2018

Please cite this article as: Fiorentino, A., Cucciniello, R., Di Cesare, A., Fontaneto, D., Prete, P., Rizzo, L., Corno, G., Proto, A., Disinfection of urban wastewater by a new photo-Fenton like process using Cu-iminodisuccinic acid complex as catalyst at neutral pH, *Water Research* (2018), doi: 10.1016/j.watres.2018.08.024.


Flow cytometry
Bacterial cells
Aggregated cells
Secondary treated
wastewater
UV-C/Cu-IDS/H$_2$O$_2$
Vs Fenton and photo-
Fenton (like) processes
(UV-C/Cu/H$_2$O$_2$
Cu/H$_2$O$_2$
UV-C/Fe/H$_2$O$_2$
Fe/H$_2$O$_2$)
Reuse
Disposal
E. Coli – plate count

Disinfection of urban wastewater by a new photo-Fenton like process using Cu-iminodisuccinic acid complex as catalyst at neutral pH

Antonino Fiorentino[1,2], Raffaele Cucciniello[3], Andrea Di Cesare[1,4], Diego Fontaneto[1], Prisco Prete[3], Luigi Rizzo[2,*], Gianluca Corno[1], Antonio Proto[3]

[1]Microbial Ecology Group, National Research Council of Italy, Institute of Ecosystem Study - Largo Tonolli 50, 28922 Verbania, Italy

[2]Department of Civil Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy.

[3]Department of Chemistry and Biology "Adolfo Zambelli", University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy.

[4]Department of Earth, Environmental, and Life Sciences, University of Genoa, 16132, Genoa, Italy.

*Corresponding author: Tel.: +39089969334; fax: +39089969620

E-mail address: l.rizzo@unisa.it

Abstract

Photo-Fenton process is among the most effective advanced oxidation processes (AOPs) in urban wastewater treatment and disinfection, but its application as tertiary treatment at full scale has not been a feasible/attractive option so far because optimum conditions are typically achieved under acidic pH. In this work a new photo Fenton like process (UV-C/H_2O_2/IDS-Cu) using iminodisuccinic acid (IDS)-Cu complex as catalyst, was compared to other processes (UV-C/H_2O_2/Cu, UV-C/H_2O_2/Fe, H_2O_2 and UV-C) in urban wastewater disinfection. Since this is the first time that IDS-Cu complex was isolated and used as catalyst, preliminary tests to evaluate the mineralization of a model compound (phenol, 25 mg L^{-1} initial concentration) in water by UV-C/H_2O_2/IDS-Cu were carried out. Almost complete mineralization of phenol (95%) was observed after 60 min treatment, being the process more effective than all other investigated AOPs (Fenton and photo-Fenton processes). This process was also proven to be more effective in the inactivation of *E. coli* (complete inactivation (3.5 log units) in 10 min) at natural pH (7.8±0.5) in real wastewater, than the other processes investigated. Unlike of what observed for *E. coli* inactivation, the investigated processes only partially inactivated total bacterial population (from 18% for UV-C to 43% for UV-C/H_2O_2/Cu), according to flow cytometry measurements. In particular, Cu based photo-Fenton processes resulted in the higher percentage of inactivated total cells, thus being consistent with the results of *E. coli* inactivation. It is worthy to note that, as H_2O_2 was decreased, UV-C/H_2O_2/Cu-IDS was more effective than UV-C/H_2O_2/Cu process. Moreover, the formation of small and large clusters decreased in the presence of Cu and Cu-IDS complex, and process efficiency improved accordingly; these results show that Cu based AOPs can more effectively disaggregate clusters, thus making disinfection process more effective than Fe based AOPs.

Keywords: advanced oxidation processes, *Escherichia coli*, flow cytometry, homogeneous photocatalysis, wastewater disinfection,

1. Introduction

Disinfection of wastewater is typically achieved by chemical (e.g., chlorine, peracetic acid) or physical (UV-C radiation) processes in urban wastewater treatment plants (UWTPs) (Di Cesare et al., 2016). Among disinfection processes, chlorination is the most widely used in UWTPs, but the formation of hazardous disinfection by-products (DBPs), such as trihalomethanes, haloacetic acids and nitrogenous disinfection by-products (N-DBPs) (Lee et al., 2007) results in environmental and public health concerns. Accordingly, peracetic acid (Antonelli et al., 2013; Formisano et al. 2016) and UV-C radiation (Di Cesare et al., 2016) are increasingly replacing chlorine in urban wastewater disinfection. However, although these treatments can effectively inactivate bacteria to meet standards for effluent disposal into the environment, they fail meeting stringent standards such as those for wastewater reuse, at least under typical operating conditions in UWTPs. Therefore, in the last years advanced oxidation processes (AOPs), among which UV/H_2O_2, photo-Fenton and TiO_2 photocatalysis, have been investigated in wastewater disinfection. Due to the production of highly oxidizing reactive oxygen species, such as hydroxyl radicals (HO•), they were found to be not only effective in the inactivation of a wide range of waterborne pathogens (Gárcia-Fernández et al. 2012) but also in the removal of a wide range of micropollutants (Klamerth et al., 2010; Ferro et al., 2015). Moreover, AOPs seem to be more effective than chlorination to reduce bacterial regrowth after disinfection (Fiorentino et al., 2015), which is a primary issue in wastewater reuse practices, where disinfected wastewater may be stored for hours/days before crops irrigation. Among AOPs, the photo-Fenton (UV/H_2O_2/Fe) process demonstrated the higher potential in the inactivation of bacteria, but its application to wastewater disinfection at full scale has not been a feasible/attractive option so far because optimum conditions are typically achieved under acidic pH (Ferro et al., 2016). As matter of fact, as pH increases, iron precipitates and it is no longer available for the reaction with H_2O_2. Therefore, in the recent years a number of different approaches have been proposed to improve the performance of photo-Fenton process. In particular, when photo-Fenton has been investigated as tertiary treatment of urban wastewater to remove contaminants of emerging concern (CECs), typically occurring at low concentrations (from a few to hundreds ng/L), as well as to inactivate bacteria, process operation under mild conditions (pH 5-6) resulted in a good efficiency (Klamerth et al., 2010; Rodríguez-Chueca et al., 2014). Other possible approaches include the use of heterogeneous and homogeneous (photo) Fenton-like processes. In particular, in heterogeneous (photo) Fenton-like processes Fe^{2+} is replaced by a solid catalyst, while homogeneous processes include a combination of other metal ion(s)/metal ion-organic ligand (Wang et al., 2016). In homogeneous (photo) Fenton-like processes Fe^{2+} is replaced by other metals (namely Cu^{2+}, Mn^{2+}, Co^{2+}, and Ag^{+}), possibly combined to organic or inorganic ligands to form complexes and/or to stabilize the metals over a wide pH range (Wang et al., 2016). Different ligands such as nitriloacetic acid (NTA), ethylenediaminetetracetic acid (EDTA), oxalic acid, tartaric acid (De Luca et al., 2014) and ethylenediamine-N-N'-disuccinic acid (EDDS) (Papoutsakis et al., 2015), have been successfully tested in tertiary treatment of municipal wastewater so far. Indeed, the research and the design of new bio-degradable and active ligands following the benign-by-design approach (Cucciniello et al., 2016) is an active task of research (Ricciardi et al., 2017). Herein, the use of iminodisuccinic acid (IDS) as metals chelating agent for photo-Fenton process has been investigated for the first time. IDS belongs to the class of percarbossilic aminoacids, such as EDDS, and it is a pentacordinating compound (Wu et al., 2015). IDS is fully bio-degradable in fumaric acid and ammonia rather than EDDS that show a percentage of biodegradability depending on the isomers ([S,S']=90%; [R,R']= not biodegradable; [S,S']+[R,R']=35%) (Groth, 1998). In this study, a Cu-IDS complex was synthesized, characterized, and applied as active catalyst for photo-Fenton disinfection process in municipal wastewaters. Preliminarily, the efficiency of photo-Fenton with Cu-IDS complex was tested in the removal of a model compound (phenol) from water and compared to a classic photo-Fenton (UV-C/Fe/H_2O_2), to UVC/H_2O_2, and to a non-iron photo-Fenton using copper sulphate. Subsequently, the process was investigated as municipal wastewater disinfection system and its effect on complex microbial communities was assessed in terms of bacterial cell abundance and size distribution (single cells, cell clusters) through flow cytometry as well as in terms of *Escherichia coli* inactivation.

2. Materials and methods

2.1 Wastewater sample

Wastewater samples were collected from the effluent of the biological process (activated sludge), just upstream of the disinfection unit (currently employing UVC) of an UWTP located in Novara (Northern Italy). Wastewater sample was stored at 4–6 ◦C and used within the same day. Samples were collected in sterilized 1 L amber glass bottles. The average values of the main gross parameters were: pH 7.65 ± 0.15, BOD_5 14 ± 1.0 mg L^{-1}, COD 30.4 ± 1.9mg L^{-1}, TSS 37.35 ± 2.15 mg L^{-1}, redox-potential 65.40 ± 3.1mV, conductivity 1115 ± 35 $mScm^{-1}$.

2.2 Chemicals

Maleic anhydride (CAS 108-31-6) 95 wt %, copper (II) sulphate pentahydrate ($CuSO_4$*5H_2O) (CAS 7758-99-8), ammonia solution (CAS 1336-21-6) 30 %, sodium hydroxide (CAS 1310-73-2), phenol (C_6H_5OH) (CAS 108-95-2) and H_2O_2 (CAS 7722-84-1) (30 wt%) were purchased from Sigma Aldrich (Saint Louis, MO, USA) and used without further purification.

2.3 Cu-IDS catalyst preparation and characterization

IDS sodium salt was synthesized by a two-step reaction that first involves the formation of sodium maleate through maleic anhydride hydrolysis in NaOH solution followed by the reaction with ammonia (Figure SI1) (Groth, 1998). In detail, 22.5 g of sodium hydroxide were dissolved in 50.0 mL of distilled water and heated to 70°C before the addition of 39.3 g of maleic anhydride, than the temperature was increased up to 100°C. 23.2 mL of ammonia solution was added and the temperature was increased up to 110°C for 18 h. Water was then removed by rotary evaporator and the obtained solid was dried at 120°C overnight. IDS yield was determined by means of UV-VIS spectrophotometry using a Varian Cary-50 spectrophotometer using Cu^{2+} to form the corresponding Cu-IDS complex with a maximum of absorbance at 710 nm. The calibration was performed by preparing four standards of Cu-IDS in the range 3.0 – 8.0 mM, using IDS (Baypure CX100®) purchased from BASF.

Copper (II)-IDS complex was prepared by dissolving a proper amount of copper sulphate and IDS in distilled water to obtain a 1:1 moles ratio for Cu^{2+}: IDS. Cu-IDS complex was then purified on a silica column using distilled water. The obtained solid was then dried at 120°C overnight.

Elemental analysis (EA) were performed using a Thermo EA 1112 (CHNS/O) instrument. Room temperature NMR spectra were recorded on a Bruker AVANCE 400 NMR. The chemical shifts were reported in δ (ppm) referenced to $SiMe_4$ and 5 mg of the complex in 0.5 mL of solvent were used for each experiment.

2.4 *Experimental set-up of photo Fenton and control tests*

Preliminary experiments to evaluate the effect of the photo-Fenton like process (UV-C/Cu-IDS/H_2O_2) on phenol were carried out in a 1.0 L cylindrical glass reactor (5.0 cm in diameter), completely covered with aluminium paper and filled in with the 500 mL of phenol (deionized) water solution. Process efficiency was evaluated in terms of phenol mineralization through total organic carbon (TOC) removal. The reactor was placed in a water bath to control the temperature at 25 °C during the experiments. The solution in the reactor was stirred continuously. The UV-C 16 W lamp (Sankyo Denky G10T5L, Japan) was located in vertical position at the centre of the glass reactor. Disinfection tests were carried out with a rotor engine based reactor, where twelve 0,1 L cylindrical glass vessels were placed at 5 cm from the UVC lamp. This experimental set-up was adopted to compare in triplicate and simultaneously the different disinfection processes on real wastewater. Furthermore, a negative control (cylindrical glasses covered with aluminium paper) for each photo driven AOP was performed too.

2.4.1 Photo-Fenton like tests for phenol removal

In order to identify the best H_2O_2/Cu-IDS ratio for photo-Fenton like process, fixed concentration of H_2O_2 (2.94 mM) and different concentration of Cu-IDS (from 0.016 to 0.5 mM) were investigated. According to the results of these preliminary tests, the operating conditions selected for the subsequent tests for phenol removal in terms of H_2O_2 concentrations were 1.470 and 29.399 mM, maintaining a constant Cu/IDS ratio .

2.4.2 Photo-Fenton like tests for wastewater disinfection

As process efficiency in terms of phenol degradation was successfully evaluated, disinfection tests were performed with lower H_2O_2 concentrations (0.735 mM (A) and 1.470 mM (B)) in order to keep residual concentration as low as possible. These concentrations of H_2O_2 were used for photo-driven AOPs tests by coupling the oxidant with Cu-IDS complex (0.01 and 0.02 mM), copper sulphate (as source of Cu^{2+}, 0.008 and 0.016 mM) and ferrous sulphate (as source of Fe^{2+}, 0.018 and 0.035mM). UVC/H_2O_2 process was also investigated for comparison. The higher concentrations (subsequently referred to as B condition) of ferrous sulphate, copper sulphate and Cu-IDS complex, were selected to meet the regulatory limits (2 mg L^{-1} for Fe and 0.1 mg L^{-1} for Cu) set by Italian regulation for UWTPs effluent disposal in surface water.

2.5 Analytical measurements

H_2O_2 concentration was determined by a colorimetric method based on the use of titanium (IV) oxysulphate (Riedel-de Haën, Germany), which forms a stable yellow complex with H_2O_2 detected by absorbance measurements at 410 nm. Absorbance was measured using a spectrophotometer UV/VIS Lambda 23 (Perkin Elmer). The signal was read with reference to a H_2O_2 standard in distilled water. Absorbance measurement was linearly correlated with H_2O_2 concentration in the range 10-1000 mg L^{-1}. The residual concentrations of Cu and Fe were measured according to the Standard Methods (1998) by atomic absorption (Perkin Elmer AAnalyst 100, Wellesley, MA, USA). TOC concentration was measured as difference between total and inorganic carbon concentrations, by a TOC analyzer HiPer TOC SA (Thermo Scientific, 168, Waltham, MA, USA). An aqueous solution of phthalic acid (2.5 mM) and tris(hydroxymethyl)aminomethane (2.4 mM) were used as the mobile phase at a flow rate of 1.5 mL min $^{-1}$ under isocratic conditions.

2.6 Microbiological analysis

Catalase was added to wastewater samples before microbiological analysis in order to remove residual H_2O_2. 1 mL samples were mixed with 20 μL of 2300 U mg^{-1} bovine liver catalase at 0.1 g L^{-1} (Sigma-Aldrich, USA). H_2O_2 and catalase at these concentrations have been demonstrated to have no detrimental effects on *E.coli* viability (García-Fernández et al. 2012).

Cell abundance and size distribution were measured by flow cytometry (Accuri C6, BD Biosciences) using 2 mL aliquots from each integrated sample. 0.5 mL aliquots were stained with SYBR Green I (ThermoScientific Inc.) solution (1%) for 15 mins in the dark and then processed at the cytometer, by setting in the cytograms a minimum of 2×10^6 events within the gate designed for single bacterial cells, and 5×10^2 events in the gate designed for >3 cells clusters (Corno et al. 2013). Gates design and cells count per mL were performed with the Accuri C6 resident analysis software (BD Biosciences). Standard plated counting method was used through 5-fold serial dilution in PBS after an incubation period of 24 h at 44°. Volume of 100 μL were plated on Tryptone Bile X-glucuronide Agar (TBX) (Sigma Aldrich, Saint Louis, MO, USA). When very low concentrations of *E.coli* were expected, 50 or 500 μL samples were spread onto agar medium. The detection limit of this experimental method was found to be 2 CFU mL^{-1}.

2.7 Statistical analysis

To address the effect of Cu-IDS and H_2O_2 on TOC, a linear model was used. Six values were tested, all at the same optimal H_2O_2/Cu-IDS ratio previously identified. The highest concentration was at 0.40 mM for Cu-IDS and 29.39 mM for H_2O_2, whereas the other concentrations were at a 5, 10, 20, 50, and 75% of the highest concentration. Analysis of Variance (ANOVA) test for model fit was used to identify whether a linear or a quadratic relationship with concentration could fit better the data. The analyses were performed including also differences between the two times of exposition, 20 and 60 minutes.

A Liner Model (LM) was used to test whether the performance in *E. coli* inactivation Vs time was significant, and whether it was significantly different between methods. ANOVA tests were performed to identify: (1) whether the performance in TOC removal was different between the eight treatment methods and at different times of exposure (20 or 60 minutes), (2) whether the performance in the removal of active and inactivated cells was different, and (3) whether the number of small clusters and aggregates was affected by differences among the investigated methods.

Tukey Honestly Significant Difference tests were performed, when necessary, to identify which methods were different from the others. All statistical tests were performed in R 3.3.3. (R Core Team, 2017)."

3. Results and discussion

3.1 Cu-IDS catalyst characterization

^{13}C-NMR spectroscopy confirmed the formation of the desired product (Figure SI2, in SI file). The results from UV-VIS spectrophotometry characterization showed that the obtained IDS yield is 55 wt %. Cu(II)-IDS complex is obtained by the selective reaction between Cu(II) salt and the mixture containing IDS. Cu-IDS was purified using a silica column and characterized by means of ^{13}C-NMR spectroscopy, elemental analysis (C/H/N/S/O determination) and atomic absorption (Cu and Na) that confirmed the high purity of the obtained compound (Figure 1).

Figure 1

The stability of the Cu-IDS complex was tested toward temperature and pH in order to evaluate its applicability as active catalyst for Fenton and photo-Fenton based processes in wastewater treatment and disinfection. The results from stability tests showed a high stability of the complex in an extended range of pH (2-8) (Figure SI3, in SI file) and temperature (20-80°C) (Figure SI4, in SI file). At pH value lower than 2 the complex seems to be not stable due to the ligand degradation (Groth, 1998).

3.2 Degradation of phenol by advanced oxidation processes

In order to evaluate the efficiency of the new complex as effective catalyst for Fenton and photo-Fenton like process in wastewater treatment, Cu-IDS complex was investigated in the removal of phenol from water. Preliminary tests using different H_2O_2/Cu-IDS ratios were performed at 20 min treatment time (2.94 mM of H_2O_2 and 0.53 mM of phenol initial concentration, pH 6) to find the optimum condition in terms of TOC removal. The optimum ratio was found to be 75, when phenol mineralization resulted in 26% TOC removal after 20 min treatment (Figure 2).

Figure 2

As optimum H_2O_2/Cu-IDS ratio was established, six different concentrations of Cu-IDS (from 0.02 mM to 0.40 mM) and H_2O_2 (from 1.47 mM to 29.39 mM) were investigated. The effect on TOC removal was better explained when including a quadratic term of the concentration of H_2O_2/Cu-IDS (ANOVA: $F=162.11$, $p<0.0001$). The higher mineralization rates were observed after 60 min treatment (Figure 3, Table SI1) and the concentration of H_2O_2/Cu-IDS was positively related to TOC removal in a non-linear relationship, with slightly differential trajectories between 20 and 60 minutes (Table SI1). As H_2O_2 and Cu-IDS initial concentrations were increased over 14.70 mM and 0.02 mM, respectively, no significant change in TOC removal was observed after 60 min treatment.

Figure 3

Moreover, UV-C/H_2O_2/Cu-IDS process was compared to (i) iron (H_2O_2/Fe^{2+}), copper (H_2O_2/Cu^{2+}) and Cu-IDS (H_2O_2/Cu-IDS) based Fenton, (ii) iron (UV-C/H_2O_2/Fe^{2+}), copper (UV-C/H_2O_2/Cu^{2+}) and Cu-IDS (UV-C/H_2O_2/Cu-IDS) based photo-Fenton, as well as to (iii) UV-C/H_2O_2 processes (H_2O_2 as standalone process was also investigated as control) (Figure 4). Treatment time affected TOC removal with higher efficiency at 60 minutes (ANOVA: $F_{1,32}=1943.9$, $p<0.0001$). The eight treatment methods differed among each other ($F_{7,32}=3094.5$, $p<0.0001$), and their efficiency differed between times ($F_{7,32}=72.3$, $p<0.0001$).

Tukey test revealed that iron, copper and Cu-IDS based Fenton did not differ among each other and from simple H_2O_2 treatment at 20 minutes, and that iron and copper based Fenton had similar effects at 60 minutes. As expected, photo Fenton processes resulted in higher efficiencies compared to Fenton processes. In particular photo Fenton processes efficiencies were in the following order: UV-C/H_2O_2/Cu-IDS>UV-C/H_2O_2/Cu^{2+}>UV-C/H_2O_2/Fe^{2+}. UV-C/H_2O_2 process resulted in a lower efficiency compared to photo Fenton processes but in higher efficiency compared to Fenton processes.

Figure 4

The initial pH of the solution was 5.7, but during the process the pH decreased to 3.7 due to the formation of oxidation by-products. Although phenols are typically used as model pollutants to investigate the efficiency of different AOPs, including photo-Fenton process, only a few works are available on the effect of metal-ligand complexes based photo-Fenton process on phenol. Prato-Garcia and coworkers (2009), investigated the performance of solar driven photo-Fenton process (compound parabolic collector (CPC) based photo reactor) in the treatment of phenolic aqueous solutions (185-200 mg L^{-1}) at natural pH, in the presence of ferrioxalate. The results achieved are consistent with the results of our experiment with UV-C/H_2O_2/Cu-IDS, because 85-100% COD reduction was observed after 120 min treatment (pH 5.6, oxalate 300 mg L^{-1}, H_2O_2/phenol 5.5-6.3). Monteagudo et al. (2011) also investigated ferrioxalate-induced solar photo-Fenton process effect on phenols, using a CPC reactor. In particular, aqueous solution contained a mixture of three phenolic compounds (gallic, p-coumaric and protocatechuic acids) and 94% TOC removal was achieved in 194 min under optimal conditions (H_2O_2 400 mg L^{-1}; Fe(II) 20 mg L^{-1}; $H_2C_2O_4$ 60 mg L^{-1}; phenols 20 mg L^{-1}; average solar power 35W m^{-2}).

3.3 Wastewater disinfection by advanced oxidation processes

UV-C/H_2O_2/Cu-IDS process was investigated in municipal wastewater disinfection through flow cytometry and plate count method (*E. coli* inactivation). Process efficiency was compared with UV-C/H_2O_2/Fe^{2+} and UV-C/H_2O_2/Cu^{2+} at natural pH (7.8±0.5). In order to meet the limits for residual copper and iron concentrations in UWTP effluents established by Italian regulation for wastewater reuse (1.0 mg L^{-1} for copper and 2 mg L^{-1} for iron) the metals were dosed to half of the limit. Due to the respective effects on bacterial inactivation, tests with H_2O_2 (0.735 mM, 25mgL^{-1}, condition A) and UV-C as standalone processes were also performed for comparison. *E. coli* inactivation Vs time was significant (ANOVA:

$F_{1,96}$=566.5, p<0.0001), with all treatment methods performing significantly better than dark ($F_{5,96}$=157.4, p<0.0001) (Figure 5). In particular, the best disinfection performance was observed with UV-C/H_2O_2/Cu-IDS process. A complete inactivation (3.5 log units) of the initial *E. coli* concentration ($5.1 \times 10^3 \pm 0.7 \times 10^3$ CFU mL^{-1}) was achieved in 10 min, while 15 and 20 min were necessary for UV-C/H_2O_2/Cu and UV-C/ H_2O_2/Fe processes, respectively. Unlike of photo-Fenton based processes, H_2O_2 and UV-C did not result in a complete inactivation of *E. coli* within 20 min treatment.

Figure 5

Although this is the first time that UV-C/H_2O_2/Cu-IDS process has been investigated in *E. coli* inactivation, the parallel experiments with conventional photo-Fenton make possible a comparison with the scientific literature. As matter of fact, bacterial inactivation by photo Fenton process (UV/H_2O_2/Fe) has been widely investigated and our results are really encouraging compared to the literature. It is noteworthy that most of the papers deal with solar driven photo-Fenton (Rodríguez-Chueca et al. 2014; Ferro et al. 2015; Villegas-Guzman et al., 2017). In particular, when solar photo-Fenton under mild conditions (pH 5, 20 mg H_2O_2 L^{-1}, 10 mg Fe L^{-1}) was investigated in the inactivation of *E. coli* in urban wastewater effluents, a 3-log unit decrease was observed (Rodríguez-Chueca et al. 2014). Moreover, natural iron and natural additives as complexing agents were also investigated in solar photo-Fenton process for the inactivation of *E. coli* in municipal wastewater (Villegas-Guzman et al., 2017). This new proposed green process resulted in a total inactivation of *E. coli* (6 log units in 180 min) in the presence of lime juice (5 mg Fe L^{-1}; 25 mg H_2O_2 L^{-1}; 600W m^{-2}). Lima Perini et al. (2018) investigated *E.coli* inactivation in hospital wastewater by photo-

Fenton with UV-A and UV-C as light sources, respectively; total inactivation (6 log units) was observed after 90 min treatment by UV-C photo-Fenton, but only 2 log units inactivation was achieved by UVA photo-Fenton.

Although the use of *E. coli* as microbial indicator is useful to evaluate the compliance of UWTPs' effluents with local regulations for wastewater disposal into the environment or reuse as well as to evaluate disinfection process efficiency and compare to scientific works available in the literature, it is not sufficient to understand the real impact of the process on bacterial population (Manaia et al., 2018). Accordingly, in this work an attempt to better characterize this impact was made through the comparison between the results from standard plate count method and flow cytometry to characterize cell abundance and size distribution. In this case the experiments were conducted at two different concentrations, and in particular the condition A, the same used for *E.coli* inactivation (H_2O_2 = 0.735 mM = 25 mg L^{-1}; Cu-IDS complex = 0.01 mM corresponding to 0.25 mgL^{-1} of Cu; copper sulphate = 0.008 mM corresponding to 0.25 mgL^{-1} of Cu; iron sulphate = 0.018 mM corresponding to 1 mgL^{-1} of Fe), and the condition B (H_2O_2 = 1.470mM = 50 mgL^{-1}; Cu-IDS complex = 0.02mM corresponding to 0.50 mgL^{-1} of Cu; copper sulphate = 0.016 mM corresponding to 0.5 mgL^{-1} of Cu; iron sulphate = 0.036 mM corresponding to 2 mgL^{-1} of Fe). The values of inactive, active cells and the percentage of inactive cells with respect to the total cells are showed in Fig 6. The higher efficiency in terms of cells inactivation among the photo-Fenton processes was observed for UV-C/H_2O_2/Cu (condition B). In the samples treated with Cu/H_2O_2 at two different concentration (A and B), the number of active cell decreased when compared to samples treated with Fe/H_2O_2 (respectively 3.0 and 2.9 log units $cells^{-1}$). Moreover, samples treated with Cu-IDS complex showed a similar decrease of the active cell number at the highest concentration (B) (2.95 log units $cell^{-1}$) as showed in Fig. 6A. Looking at the number of inactivated cells (Fig 6B), as absolute number photo-Fenton processes had similar results, although treatment with Cu/H_2O_2 appears to be more aligned with the results of only UVC and UVC/H_2O_2. However, the interesting result is represented by the number of inactive cells with respect to the totals (Fig. 6C). Among the lowest concentrations (A) investigated, photo-Fenton by Cu-IDS was the most performing process (37% of inactivated cells). All other treatments with low concentrations (A) resulted in a percentage of inactivated cells less than 30%. At the highest concentrations (B), the most performant treatment was the photo-Fenton process by Cu/H_2O_2 (44% of inactivated cells), immediately followed by the treatment by Cu-IDS (40%).

Figure 6

In order to better understand the behaviour of bacterial cells under the action of the different disinfection processes investigated, cytometric analysis has been also carried out to characterize cell clusters (small clusters and aggregates) (Fig 7). . The higher formation of small clusters (5.4 and 5.6 log units mL^{-1}) was observed for $UV\text{-}C/Fe/H_2O_2$ process at two different concentrations (A and B, respectively) (Fig 7A). Interestingly, Fenton process (Fe^{2+}/H_2O_2) resulted in the formation of a higher amount (5.5 log units) of small clusters compared to the $Cu\text{-}IDS/H_2O_2$ and Cu^{2+}/H_2O_2 (approximately 5.3 log units) and the remaining processes (approximately 5.1 log units). Looking at the formation of large clusters (aggregates), the higher formation was observed for Cu/H_2O_2 process (approximately 3.3 log units) (Fig. 7B). Among photo-Fenton processes $UV\text{-}C/Fe/H_2O_2$ resulted in the higher formation of aggregates (approximately 3.1 log units), for the higher H_2O_2 concentration (B). The formation of small clusters and aggregates might promote resistance among cells to the disinfection process and slow down bacterial inactivation. Even in dark experiments the cells have a great capacity to form clusters, therefore the UV-C in photo driven AOPs might be the key factor affecting the process and speeding up the inactivation kinetics. The cluster formation is the response of bacterial communities to stress such as UV light exposure (Kollu and Ormeci, 2015), predation (Corno and Jurgens, 2006), antibiotic pressure (Corno et al., 2014), and chemical disinfection (Di Cesare et al., 2016). It is worthy to mention that, when the effect of low doses of disinfectant (peracetic acid) was investigated, cluster formation drastically decreased as the dose was increased (from 0 to 25 mg L^{-1} min) (Turolla et al., 2017). According to the results of our work, the bacterial cell response depends on the various oxidative stress factors affecting the cells. The Fe based AOPs, investigated in this work, promoted the formation of clusters, decreasing bacterial inactivation efficiency, especially at the highest concentrations (B). Instead, in the presence of Cu and Cu-IDS complex, the formation of small and large clusters decreased and process efficiency improved accordingly.

Figure 7

4. Conclusions

The new photo-Fenton like process (UV-C/H_2O_2/Cu-IDS) investigated in this work was proven to be really effective in the inactivation of *E. coli* (complete inactivation (3.5 log units) in 10 min) at natural pH (7.8±0.5) in real wastewater, also compared to the other processes investigated (UV-C/H_2O_2/Cu, UV-C/H_2O_2/Fe, H_2O_2 and UV-C). Unlike of what observed for *E. coli* inactivation, the investigated processes only partially inactivated total bacterial population (from 18% for UV-C to 42% for UV-C/H_2O_2/Cu-IDS), according to flow cytometry measurements. In particular, Cu based photo-Fenton processes resulted in the higher percentage of inactivated total cells, thus being consistent with the results of *E. coli* inactivation. It is worthy to note that, as H_2O_2 was decreased, UV-C/H_2O_2/Cu-IDS was more effective than UV-C/H_2O_2/Cu process. Flow cytometry also confirmed that both Fenton and photo-Fenton processes are more effective than conventional disinfection processes (UV-C and H_2O_2, respectively) in the inactivation of total bacteria population. Moreover, the formation of small and large clusters decreased in the presence of Cu and Cu-IDS complex, and process efficiency improved accordingly; these results show that Cu based AOPs can more effectively disaggregate clusters, thus making disinfection process more effective than Fe based AOPs and other investigated processes. Most important, UV-C/H_2O_2/Cu-IDS process is really effective under real conditions in urban wastewater, thus overcoming the main obstacle (acidic pH) to the use of photo-Fenton process.

Acknowledgements

This research was supported by (i) the Italian Ministry of Environment and Protection of Land and Sea and the Italian Ministry of Foreign Affairs through the Bilateral High-Relevance Project Italy-China 2016-18 “Efficiency of different disinfection processes in the removal of antibiotic resistance determinants in experimental pilot systems and full-scale WWTPs” and (ii) University of Salerno through the project “Tertiary treatment of urban wastewater by photo driven advanced oxidation processes” (ORSA178411 - FARB2017).

References

Antonelli, M., Turolla, A., Mezzanotte, V., Nurizzo, C., 2013. Peracetic acid for secondary effluent disinfection: A comprehensive performance assessment. Water Science & Technology 68, 2638-2644.

Corno, G., Coci, M., Giardina, M., Plechuk, S., Campanile, F., Stefani, S., 2014. Antibiotics promote aggregation within aquatic bacterial communities. Frontiers in Microbiology 5, 297.

Corno, G., Jurgens, K., 2006. Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. Applied and Environmental Microbiology 72, 78-86.

Corno, G., Villiger, J., Pernthaler, J., 2013. Coaggregation in a microbial predator–prey system affects competition and trophic transfer efficiency. Ecology 94, 870-881.

Cucciniello, R., Ricciardi, M., Vitiello, R., Di Serio, R., Proto, A., Capacchione, C., 2016. Synthesis of Monoalkyl Glyceryl Ethers by Ring Opening of Glycidol with Alcohols in the Presence of Lewis Acids. ChemSusChem 9, 3272-3275.

De Luca, A., Dantas, R.F., Esplugas, S., 2014. Assessment of iron chelates efficiency for photo-Fenton at neutral pH. Water Research 61, 232-242.

Di Cesare, A., Fontaneto, D., Doppelbauer, J., Corno, G., 2016. Fitness and Recovery of Bacterial Communities and Antibiotic Resistance Genes in Urban Wastewaters Exposed to Classical Disinfection Treatments. Environmental Science & Technology, 50, 10153-10161.

Ferro, G., Fiorentino, A., Castro Alferez, M., Polo-López, M.I., Rizzo, L., Fernández-Ibáñez, P., 2016. Urban wastewater disinfection for agricultural reuse: effect of solar driven AOPs in the inactivation of a multidrug resistant E. coli strain. Applied Catalysis B: Environmental 178, 65-73.

Ferro, G., Polo-López, M. I., Martínez-Piernas, A.B., Fernández-Ibáñez, P., Agüera A., Rizzo L., 2015. Cross-Contamination of Residual Emerging Contaminants and Antibiotic Resistant Bacteria in Lettuce Crops and Soil Irrigated with Wastewater Treated by sunlight/H2O2. Environmental Science and Technology 49, 1096−11104.

Fiorentino, A., Ferro, G., Castro Alferez, M., Polo-López, M.I., Fernández-Ibañez, P., Rizzo, L., 2015. Inactivation and regrowth of multidrug resistant bacteria in urban wastewater after disinfection by solar-driven and chlorination processes. Journal of Photochemistry and Photobiology B: Biology 148, 43–50.

Formisano, F., Fiorentino, A., Rizzo, L., Carotenuto, M., Pucci, L., Giugni, M., Lofrano, G., 2016. Inactivation of Escherichia coli and Enterococci in urban wastewater by sunlight/PAA and sunlight/H_2O_2 processes. Process Safety and Environmental Protection 104, 178-184.

Gárcia-Fernández, I., Polo-López, M. I., Oller, I., Fernández-Ibáñez, P., 2012. Bacteria and fungi inactivation using Fe^{3}+/sunlight, H_2O_2/sunlight and near neutral photo-Fenton: A comparative study. Applied Catalysis B: Environmental 121-122, 20-29.

Groth, T. Preparation and use of iminodisuccinic acid salts, PCT No.: WO98/45251, 1998.

Klamerth, N., Rizzo, L., Malato, S., Maldonado, M.I., Agüera, A., Fernández-Alba A.R., 2010. Degradation of fifteen emerging contaminants at μg L−1 initial concentrations by mild solar photo-Fenton in MWTP effluents. Water Research 44, 545-554.

Kollu, K., Ormeci, B., 2015. UV-induced self-aggregation of E. coli after low and medium pressure ultraviolet irradiation. Journal of Photochemistry and Photobiology B: Biology. 148, 310-321.

Lee, W., Westerhoff, P., Croué, J., 2007. Dissolved Organic Nitrogen as a Precursor for Chloroform, Dichloroacetonitrile, N-Nitrosodimethylamine, and Trichloronitromethane. Environmental Science & Technology 41, 5485–5490.

Lima Perini, J.A., Tonetti, A.L., Vidal, C., Montagner, C.C., Pupo Nogueira, R.F., 2018. Simultaneous degradation of ciprofloxacin, amoxicillin, sulfathiazole and sulfamethazine, and disinfection of hospital effluent after biological treatment via photo-Fenton process under ultraviolet germicidal irradiation. Applied Catalysis B: Environmental 224, 761-771.

Manaia, C., Rocha, J., Scaccia, N., Marano, R., Radud, E., Biancullo, B., Cerqueira, Fortunato, G., Iakovides, I.C., Zammit, I., Kampourius, I., Vaz-Moreira, I., Nunes, O.C., 2018. Antibiotic resistance in wastewater treatment plants: Tackling the black box. Environment International 115, 312–324.

Papoutsakis, S., Miralles-Cuevas, S., Oller, I., Garcia-Sanchez, J.L., Pulgarin, C., Malato, S., 2015. Microcontaminant degradation in municipal wastewater treatment plant secondary effluent by EDDS assisted photo-Fenton at near-neutral pH: An experimental design approach, Catalysis Today 252, 61-69.

Prato-Garcia, D., Vasquez-Medrano, R., Hernandez-Esparza, M., 2009. Solar photoassisted advanced oxidation of synthetic phenolic wastewaters using ferrioxalate complexes. Solar Energy 83, 306–315.

R Core Team,2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ricciardi, M., Passarini, F., Vassura, I., Proto, A., Capacchione, C., Cucciniello, R., Cespi, D., 2017. Glycidol, a Valuable Substrate for the Synthesis of Monoalkyl Glyceryl Ethers: A Simplified Life Cycle Approach, ChemSusChem 10, 2291-2300.

Rodríguez-Chueca, J., Polo-López M.I., Mosteoa, R., Ormada, M.P., Fernández-Ibánez, P., 2014. Disinfection of real and simulated urban wastewater effluents using a mild solar photo-Fenton. Applied Catalysis B: Environmental, 150–151, 619-629.

Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998. America Public Health Association, America Water Works Association, Water Environment Federation, Washington, DC, USA.

Turolla, A., Sabatino, R., Fontaneto, D., Eckert, E.M., Colinas, N., Corno, G., Citterio, B., Biavasca, F., Antonelli, M., Mauro, A., Mangiaterra, G., Di Cesare, A., 2017. Defence strategies and antibiotic resistance gene abundance in enterococci under stress by exposure to low doses of peracetic acid. Chemosphere 18, 480-488.

Villegas-Guzman, P., Giannakis, S., Rtimi, S., Grandjean, D., Bensimon, M., Felippe de Alencastro, Torres-Palma, R., Pulgarin, C., 2017. A green solar photo-Fenton process for the elimination of bacteria and micropollutants in municipal wastewater treatment using mineral iron and natural organic acids. Applied Catalysis B: Environmental 219, 538-549.

Wang, N., Zheng, T., Zhang, G., Wang, P., 2016. A review on Fenton-like processes for organic wastewater treatment. Journal of Environmental Chemical Engineering 4, 762-787.

Wu, Q, Duan, G., Cui, Y., Sun, J., 2015. Removal of heavy metal species from industrial sludge with the aid of biodegradable iminodisuccinic acid as the chelating ligand. Environmental Science and Pollution Research 22, 1144-1150.

Figure captions

Figure 1. Elemental analysis and ^{13}C-NMR spectra of purified Cu(II)-IDS complex. (^{13}C-NMR (400 MHz, D_2O), δ: 179.9 ppm (1), 174.8 ppm (4), 59.8 ppm (3), 59.4 ppm, 40.2 ppm (2), 39,9 ppm).

Figure 2. Phenol mineralization (TOC removal) by photo-Fenton like process (UV-C/H_2O_2/Cu-IDS): optimization of H_2O_2/Cu-IDS ratio (20 min treatment time, 2.94 mM of H_2O_2 and 0.53 mM of phenol initial concentration, pH 6).

Figure 3. Phenol degradation (50 mg L^{-1} initial concentration, pH 6) by photo-Fenton like process in terms of TOC removal at 75 H_2O_2/Cu-IDS value: effect of H_2O_2 and Cu-IDS initial concentrations.

Figure 4. Phenol degradation in terms of TOC removal (50 mg L^{-1} of initial phenol concentration, pH 6.0): comparison among UV-C/H_2O_2/Cu-IDS, Fenton (H_2O_2/Fe^{2+}, H_2O_2/Cu^{2+}, H_2O_2/Cu-IDS), photo Fenton (UV-C/H_2O_2/Fe^{2+}, UV-C/H_2O_2/Cu^{2+}, UV-C/H_2O_2/Cu-IDS) and UV-C/H_2O_2 processes (H_2O_2 control test).

Figure 5. *E. coli* inactivation by photo-Fenton processes, UV-C/H_2O_2, UV-C and H_2O_2. The behaviour of *E. coli* in absence of treatment is also included (control test in dark).

Figure 6. Effect of different processes on bacterial population measured by flow cytometry: active cells (A), inactive cells (B), proportion of inactive cells (C).

Figure 7. Effect of different processes on bacterial population measured by flow cytometry: small clusters (A), large clusters (B).

Cu	C	H	N	O	Na
17.9 %	27.1 %	2.2 %	3.3 %	36.1 %	13.4 %

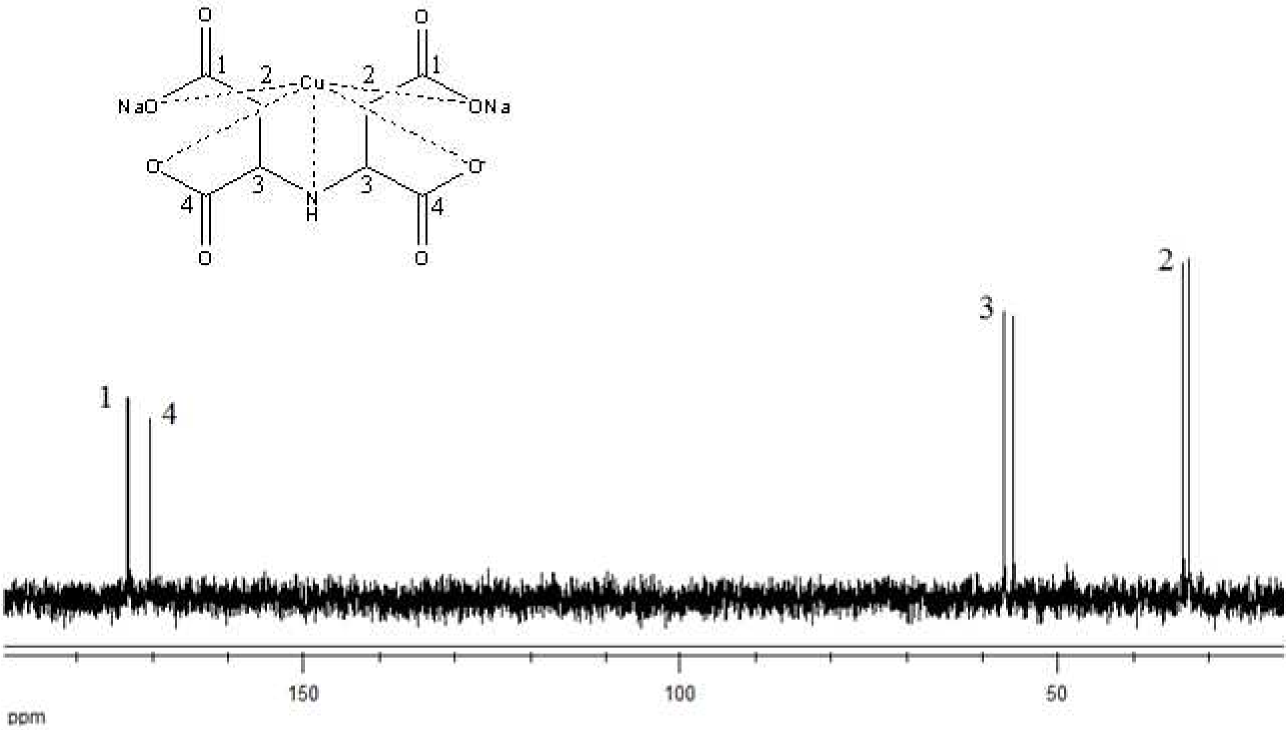


Figure 1

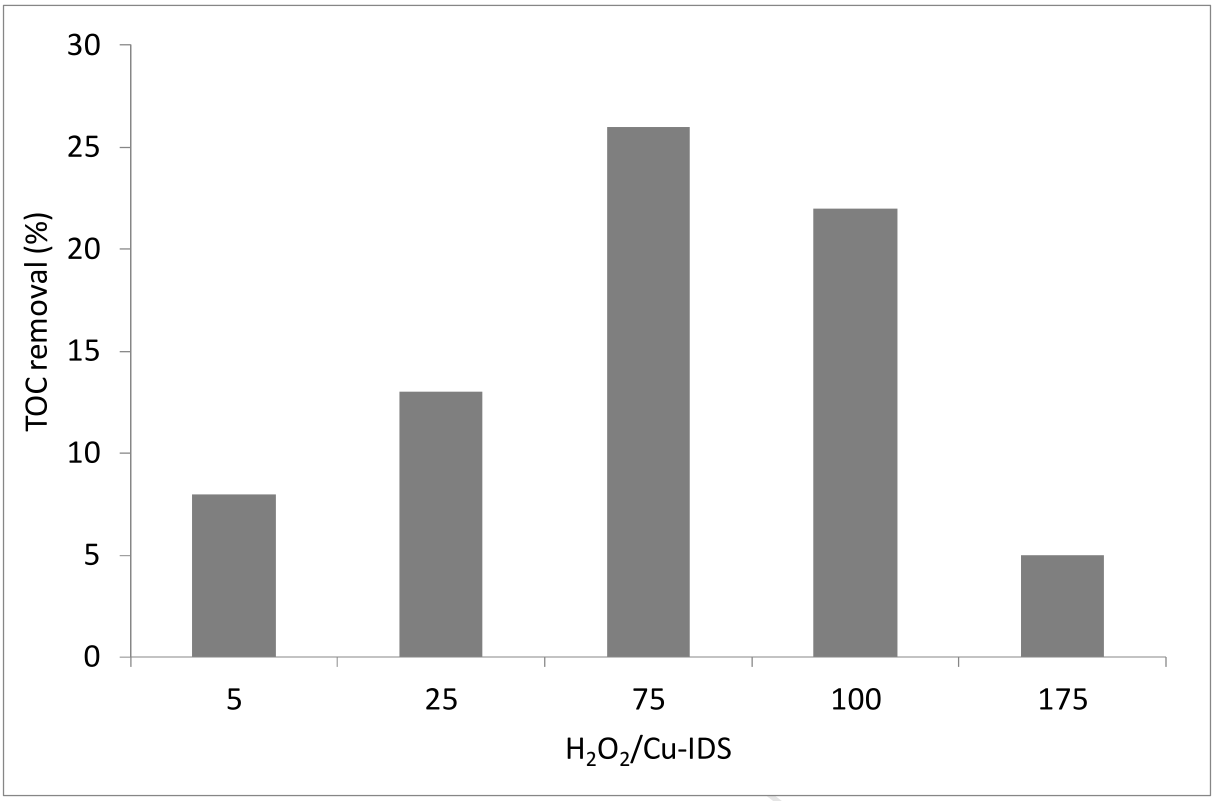


Figure 2

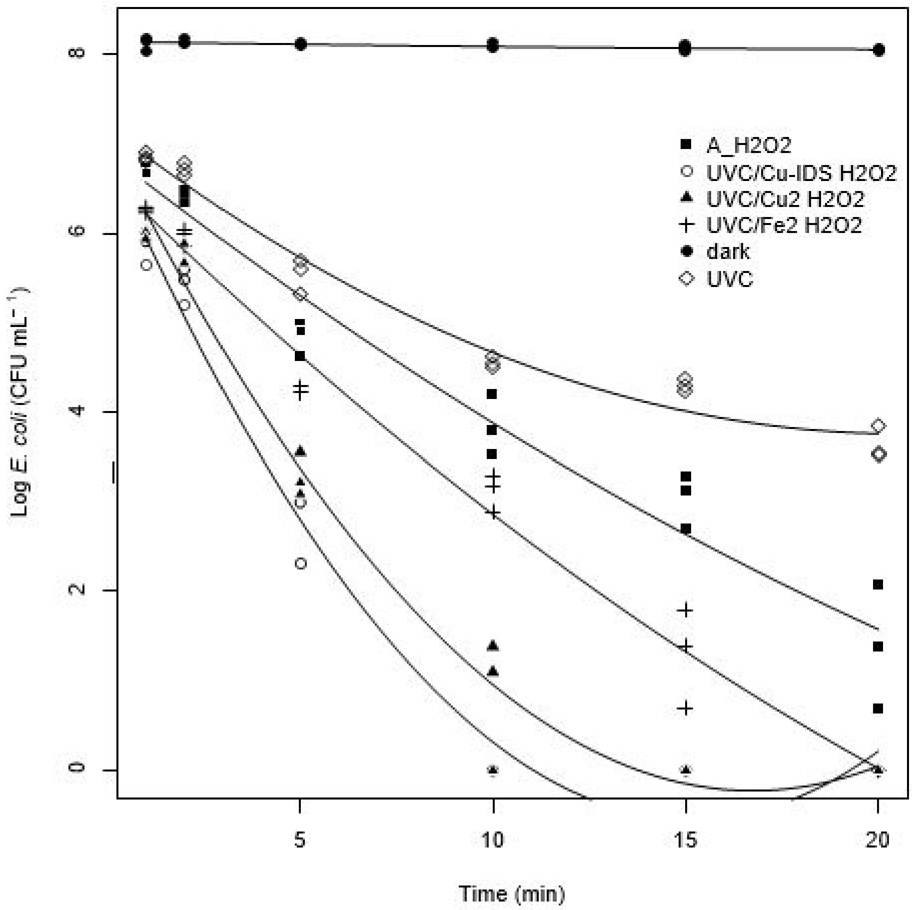

A_H2O2
UVC/Cu-IDS H2O2
UVC/Cu2 H2O2
UVC/Fe2 H2O2
dark
UVC
Log E. coli (CFU mL⁻¹)
Time (min)
0
2
4
6
8
5
10
15
20

Figure 5

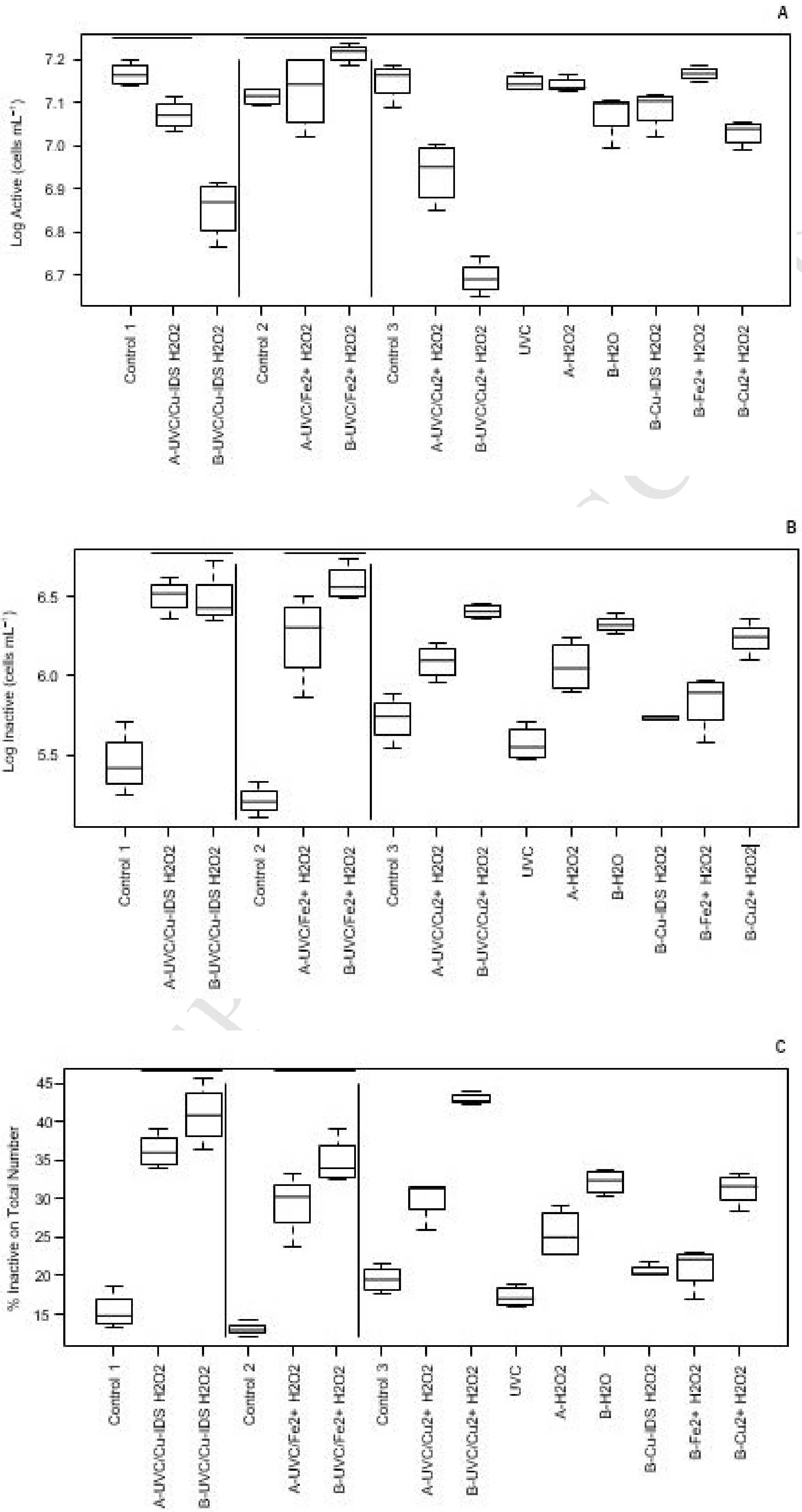
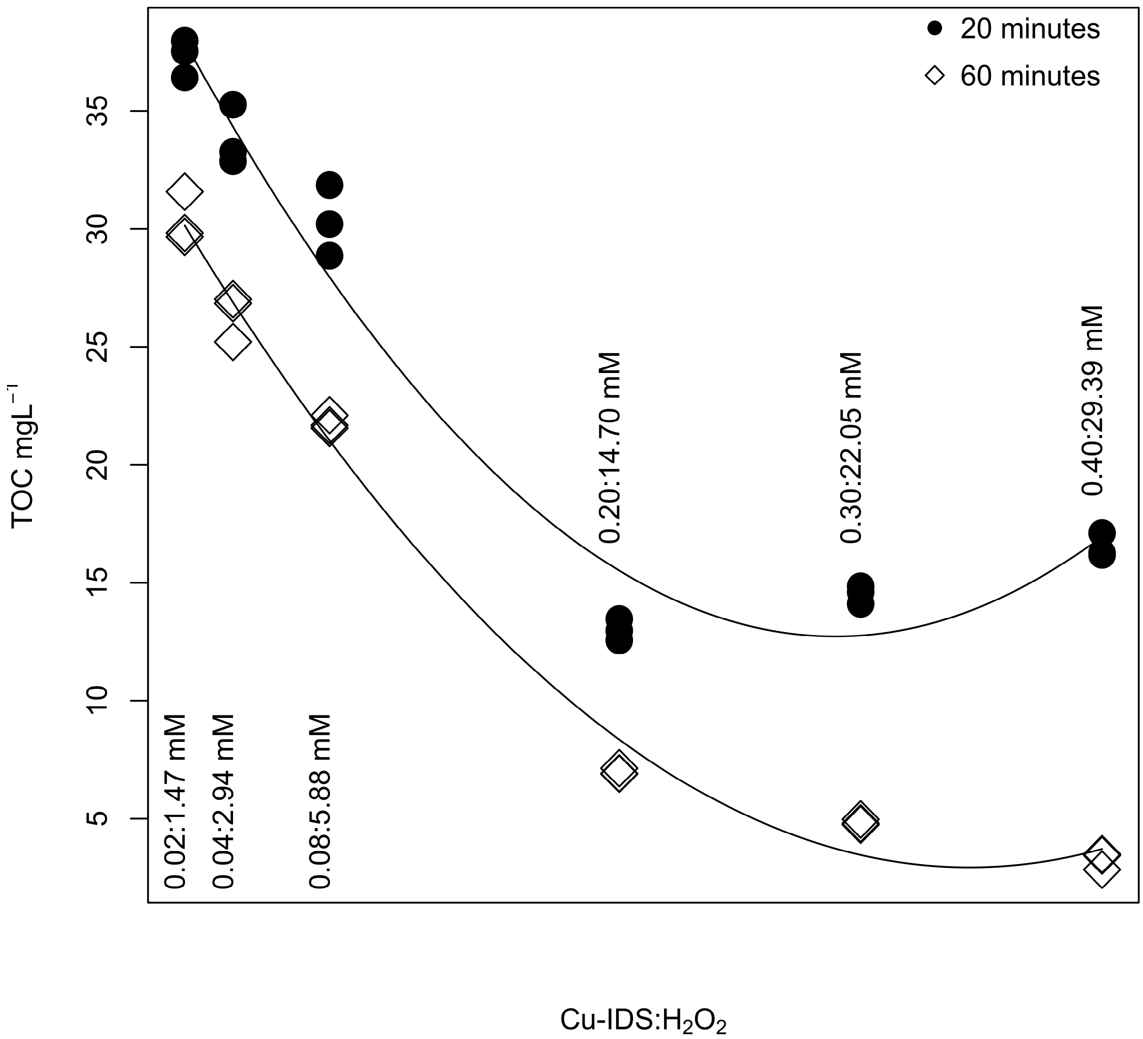
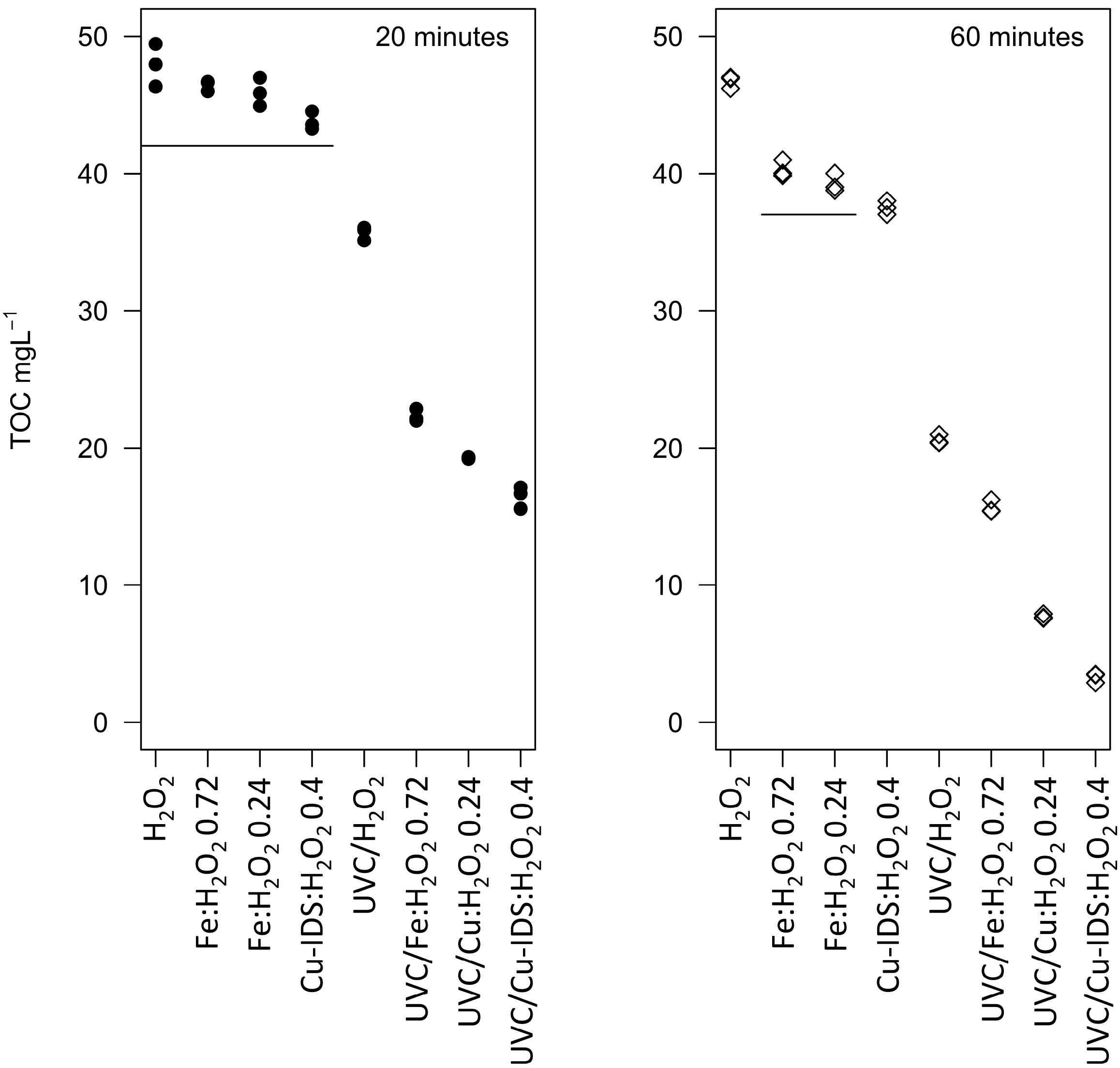
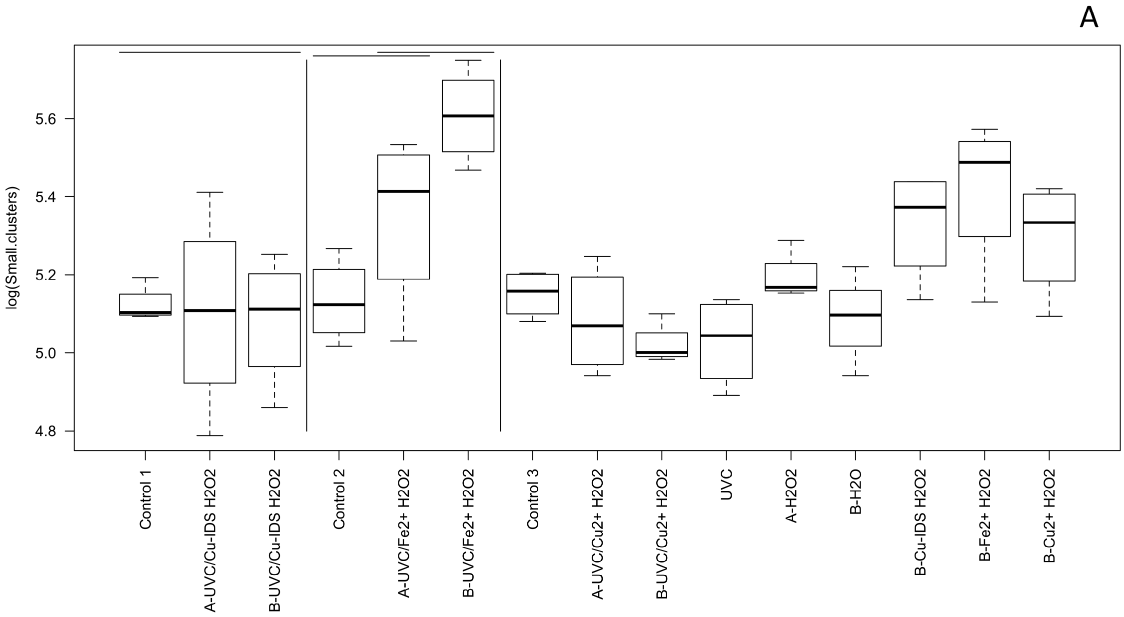
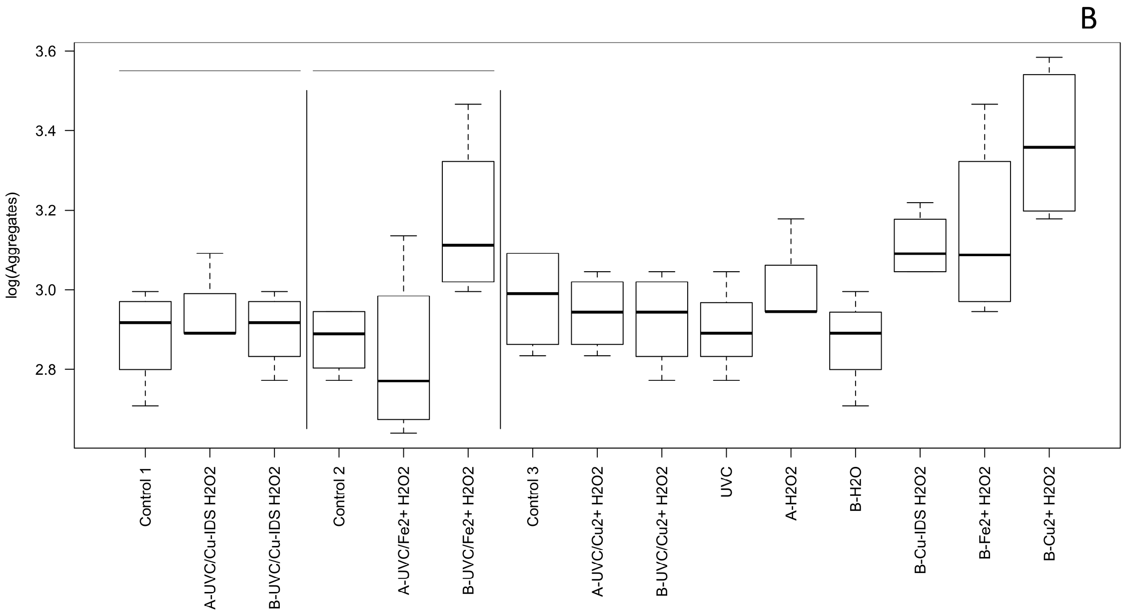

A
Log Active (cells mL⁻¹)
7.2
7.1
7.0
6.9
6.8
6.7
Control 1
A-UVC/Cu-IDS H2O2
B-UVC/Cu-IDS H2O2
Control 2
A-UVC/Fe2+ H2O2
B-UVC/Fe2+ H2O2
Control 3
A-UVC/Cu2+ H2O2
B-UVC/Cu2+ H2O2
UVC
A-H2O2
B-H2O
B-Cu-IDS H2O2
B-Fe2+ H2O2
B-Cu2+ H2O2
B
Log Inactive (cells mL⁻¹)
6.5
6.0
5.5
C
% Inactive on Total Number
45
40
35
30
25
20
15

Figure 6


20 minutes
60 minutes
TOC mgL⁻¹
35
30
25
20
15
10
5
0.02:1.47 mM
0.04:2.94 mM
0.08:5.88 mM
0.20:14.70 mM
0.30:22.05 mM
0.40:29.39 mM
Cu-IDS:H2O2


20 minutes
60 minutes
TOC mgL^{-1}
0
10
20
30
40
50
H_2O_2
Fe:H_2O_2 0.72
Fe:H_2O_2 0.24
Cu-IDS:H_2O_2 0.4
UVC/H_2O_2
UVC/Fe:H_2O_2 0.72
UVC/Cu:H_2O_2 0.24
UVC/Cu-IDS:H_2O_2 0.4


A
log(Small.clusters)
5.6
5.4
5.2
5.0
4.8
Control 1
A-UVC/Cu-IDS H2O2
B-UVC/Cu-IDS H2O2
Control 2
A-UVC/Fe2+ H2O2
B-UVC/Fe2+ H2O2
Control 3
A-UVC/Cu2+ H2O2
B-UVC/Cu2+ H2O2
UVC
A-H2O2
B-H2O
B-Cu-IDS H2O2
B-Fe2+ H2O2
B-Cu2+ H2O2


B
log(Aggregates)
3.6
3.4
3.2
3.0
2.8
Control 1
A-UVC/Cu-IDS H2O2
B-UVC/Cu-IDS H2O2
Control 2
A-UVC/Fe2+ H2O2
B-UVC/Fe2+ H2O2
Control 3
A-UVC/Cu2+ H2O2
B-UVC/Cu2+ H2O2
UVC
A-H2O2
B-H2O
B-Cu-IDS H2O2
B-Fe2+ H2O2
B-Cu2+ H2O2

Highlights

- First time that Cu-IDS complex was used as catalyst in photo-Fenton like process
- More effective than all other investigated AOPs in phenol mineralization.
- Even more effective in *E. coli* inactivation at neutral pH in real ww.
- Small and large cell clusters decreased in the presence of Cu-IDS complex
- Cu based AOPs resulted in a more effective disinfection than Fe based AOPs