

EU PhD School on AOPs

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  News Summer School 2015

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AOPs PhD School

- [Home](#)
- [Objectives](#)
- [Scientific Committee](#)
- [School Candidates](#)
- [Awarded Candidates](#)
- [Enterprises](#)
- [Contact](#)

Welcome

European PhD School on Advanced Oxidation Processes

Our goal is to promote the higher education of young researchers in the environmental applications of AOPs



EU PhD School on AOPs

- ✓ Scientific Committee: 54 members from Universities and Research Institutes of 15 EU Countries.
- ✓ PhD students: 60
- ✓ Alumni: 26



- ✓ 1st Summer School (June 15-19, Fisciano (SA), Italy): 70 participants among lecturers and PhD students, from 15 different countries.

Advances in photo driven AOPs for controlling antibiotic resistance spread from urban wastewater effluents

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“EU PhD School on AOPs” and “NEREUS” Summer School, Porto, 10-14 July 2017

Summary

- Antibiotic resistance (AR)
- AR transfer mechanisms
- AR in urban wastewater treatment plants (UWWTPs)
- Tertiary treatment in UWWTPs
- Effect of disinfection processes on AR
- Advanced oxidation processes (AOPs)
- Effect of photo driven AOPs on AR (ARB&Gs)
- Conclusions

Antibiotic resistance

The Washington Post

To Your Health

The superbug that doctors have been dreading just reached the U.S.

By [Lena H. Sun](#) and [Brady Dennis](#) May 27

For the first time, researchers have found a person in the United States carrying bacteria resistant to antibiotics of last resort, an alarming development that the top U.S. public health official says could mean “the end of the road” for antibiotics.

BBC NEWS

HEALTH

11 December 2014 Last updated at 00:29 GMT

[Fergus Walsh](#)

Medical correspondent

[More from Fergus](#)



Superbugs to kill 'more than cancer' by 2050

[COMMENTS \(564\)](#)

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

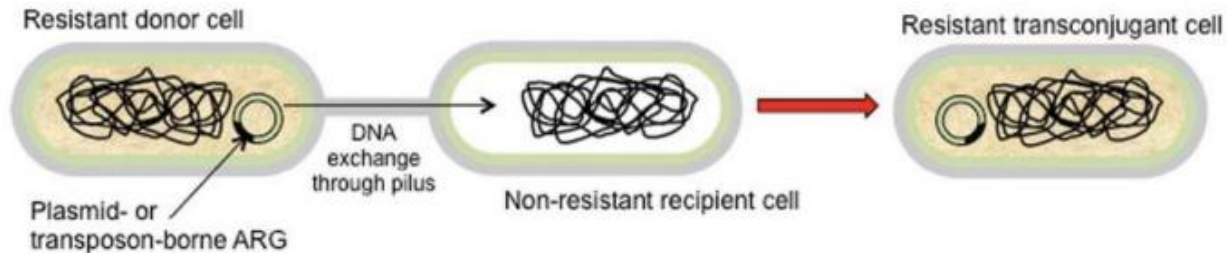
They are currently implicated in 700,000 deaths each year.

Antibiotic resistance

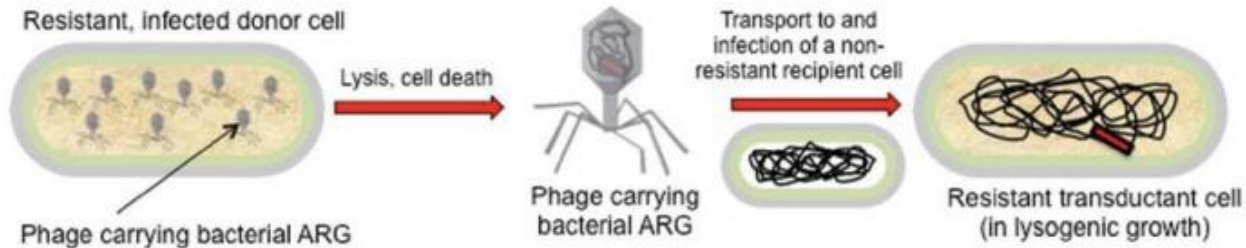
- Antibiotic resistance (AR) has become an ongoing clinical and public health issue of concern worldwide (WHO 2014).
- Depending on the scenario, failing to tackle AR treatment will mean that (Taylor et al., 2014):
 - ✓ the world population by 2050 may decrease by 11 million (if resistance rates will be successfully addressed) and 444 million (a world with no effective antimicrobial drugs) than it would otherwise be in the absence of AR.
 - ✓ the world economy would be smaller by between 0.06% and 3.10%, again depending on the scenario, and world Gross Domestic Product losses may result in a cumulative loss that ranges between \$2.1 trillion and \$124.5 trillion.

AR transfer mechanisms

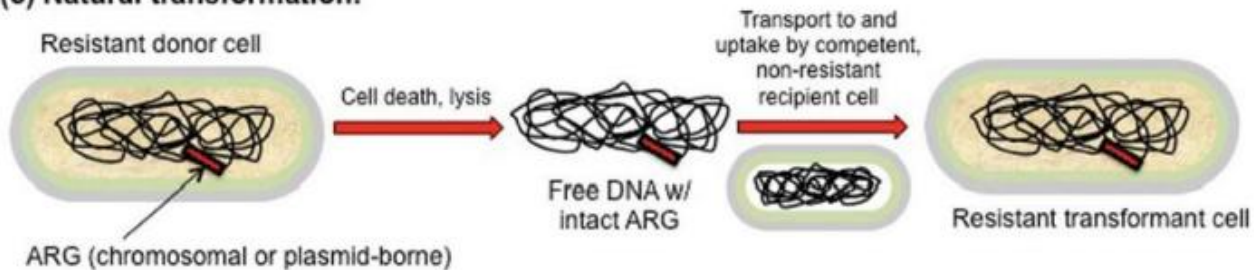
(a) Conjugation:



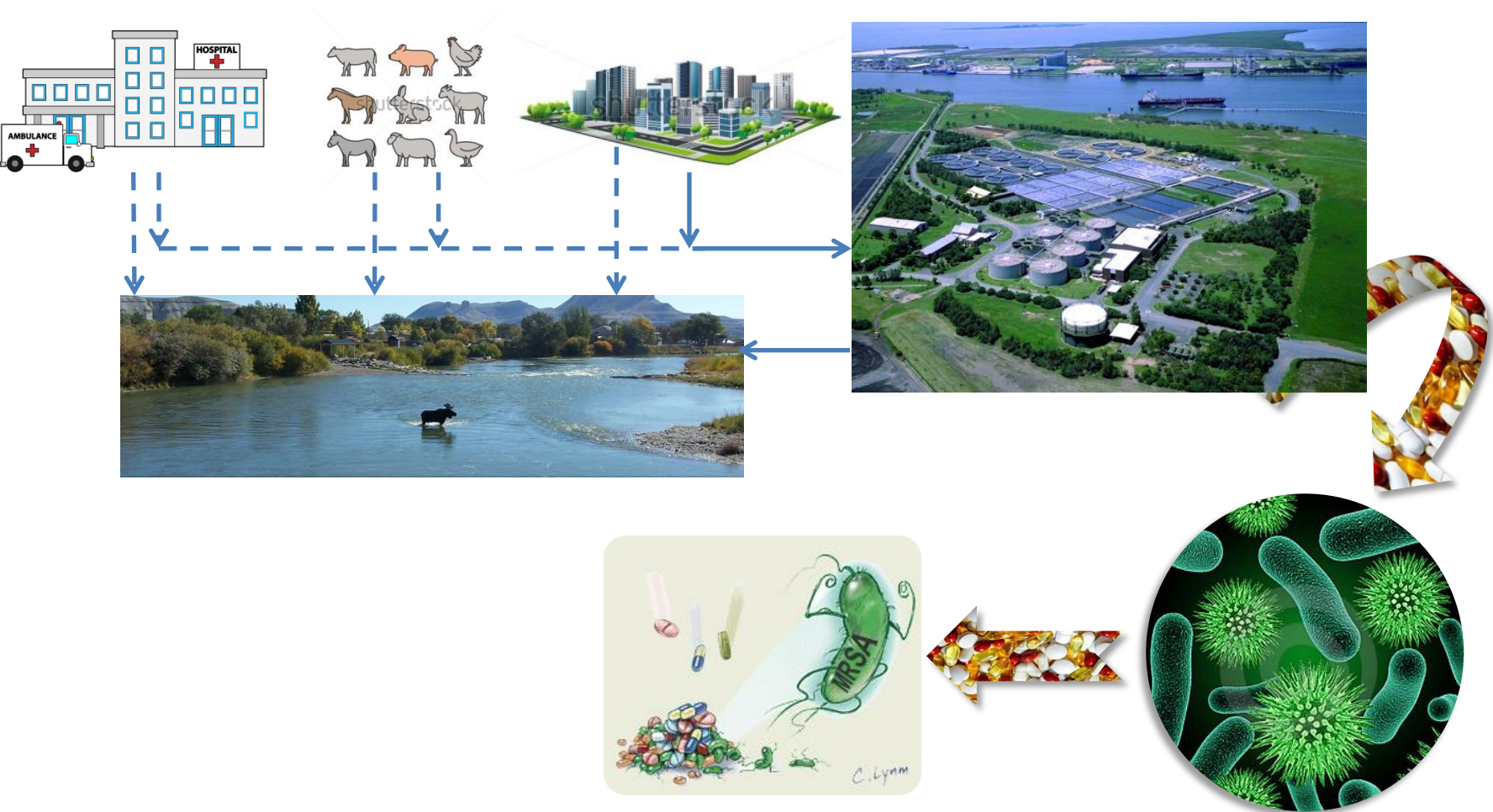
(b) Transduction:



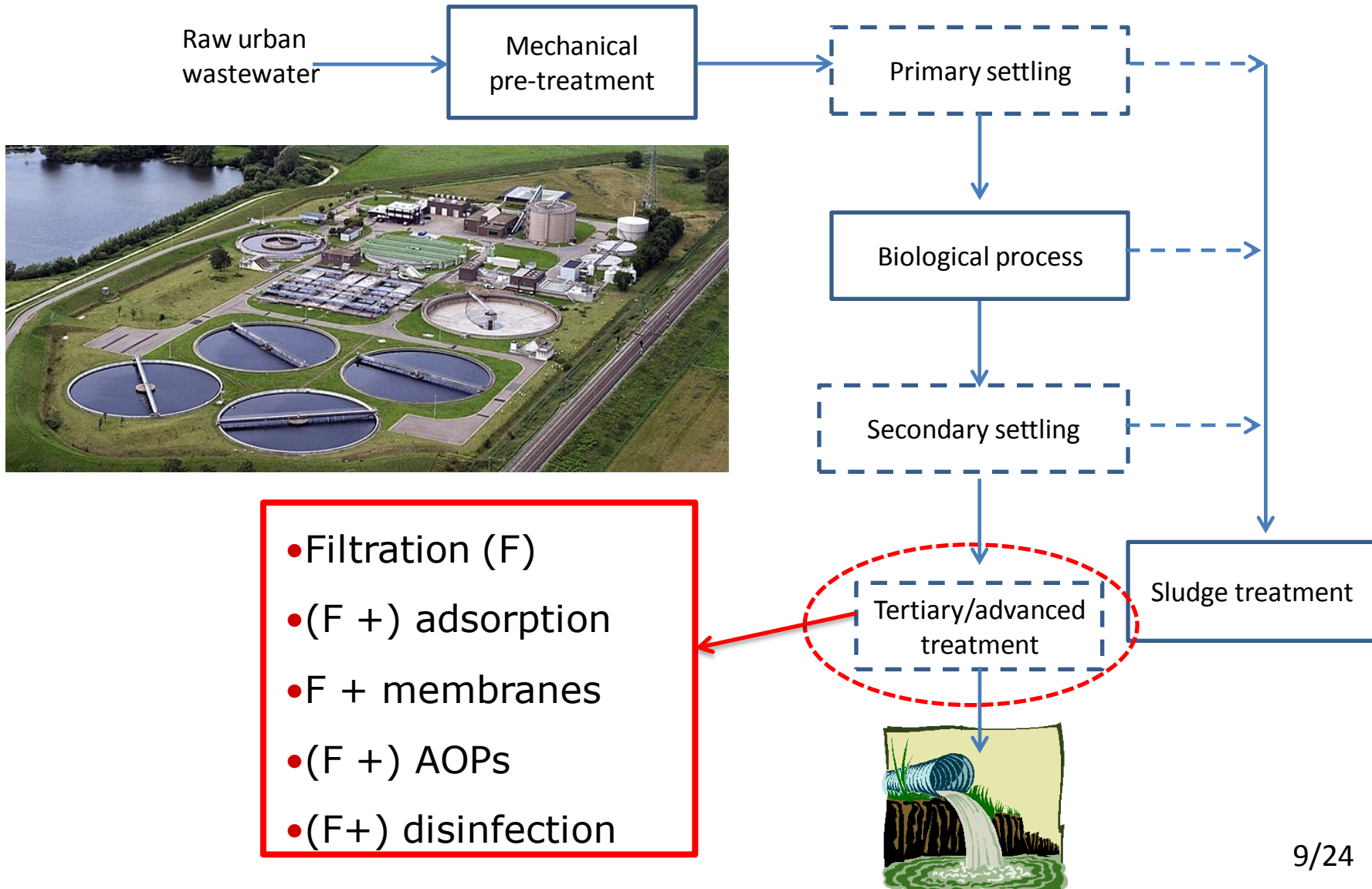
(c) Natural transformation:



Antibiotic resistance in UWWTPs



Tertiary treatment in UWWTPs



Tertiary treatment in UWWTPs

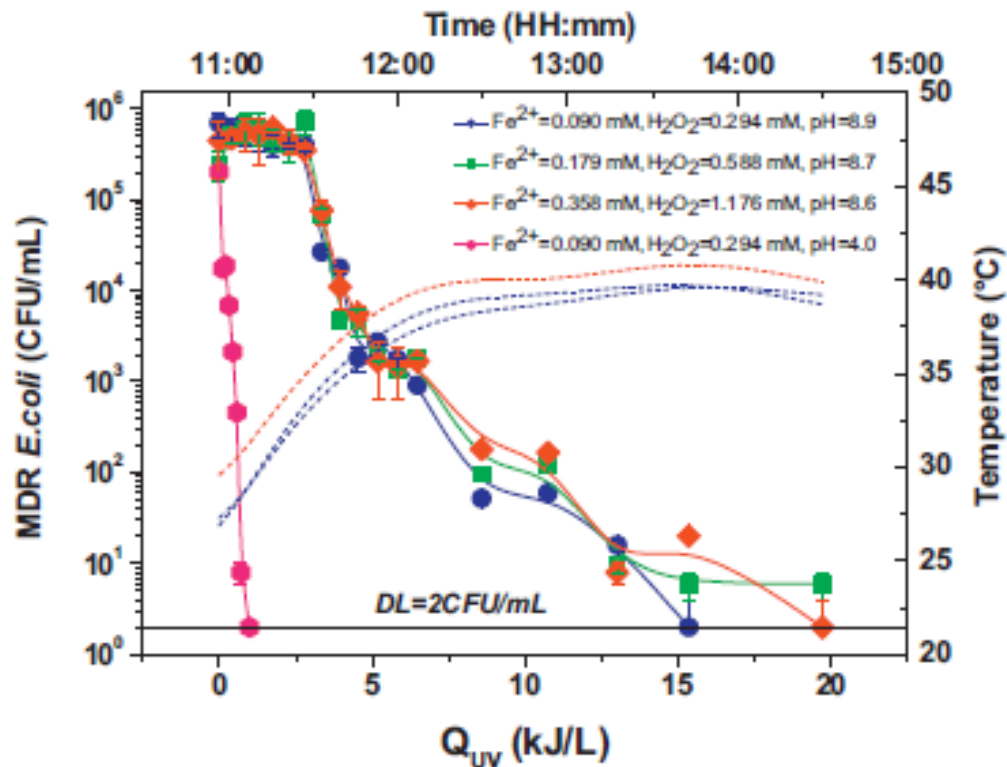
- Disinfection processes
 - ✓ Chlorination
 - ✓ UV-C radiation
 - ✓ Peracetic acid
- Physical processes
 - ✓ Rapid granular filtration
 - ✓ Membranes (UF, MF, NF)
- Physico-chemical processes
 - ✓ Adsorption (typically PAC, GAC)
- Advanced oxidation processes (AOPs)
 - ✓ Ozonation, UV/H₂O₂, photo-Fenton ... etc.

Advanced oxidation processes (AOPs)

- ... have been widely investigated in the removal of organic and inorganic contaminants from water/wastewater as well as disinfection processes;
- Among AOPs, photo driven processes, including photo-Fenton and UV/H₂O₂ as well as heterogeneous photocatalytic processes (e.g., UV/TiO₂) have been found to be effective in the inactivation of a wide range of bacteria;
- AOPs produce radical species (e.g., hydroxyl radicals (HO•)).

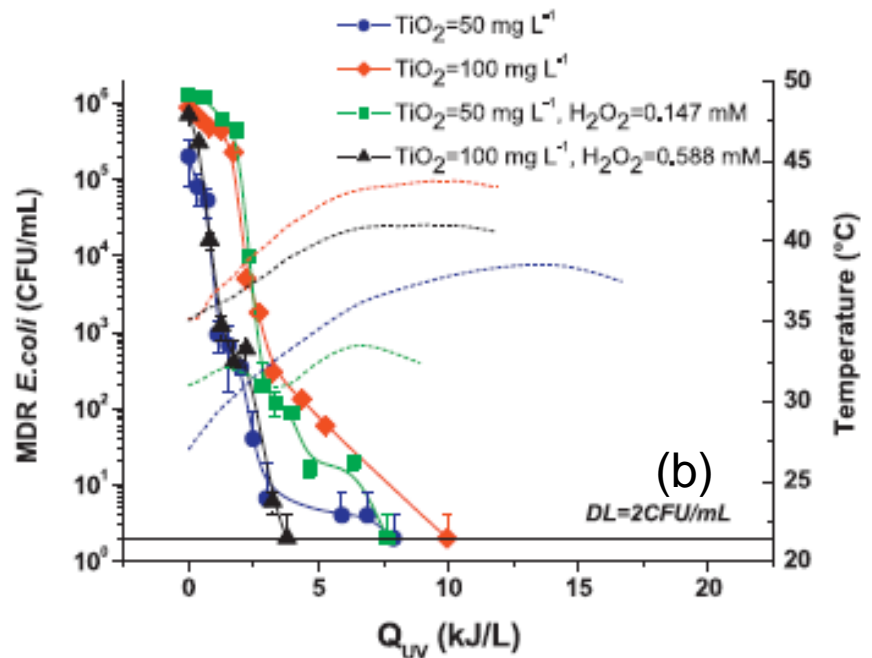
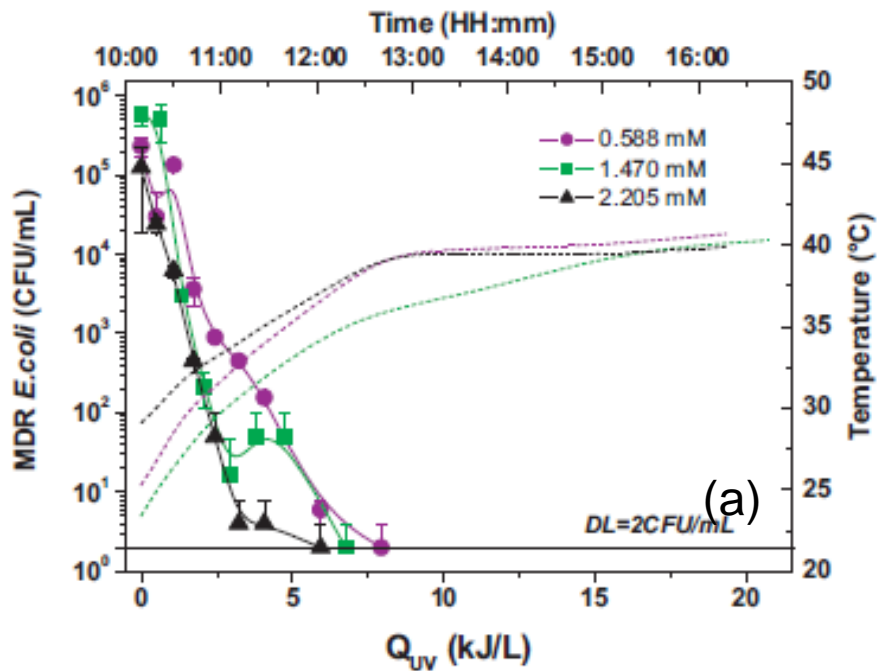
Effect of photo driven AOPs on ARB

- Effect of solar photo-Fenton on multi-drug resistant *E. coli* strain (ampicillin, ciprofloxacin, tetracycline)



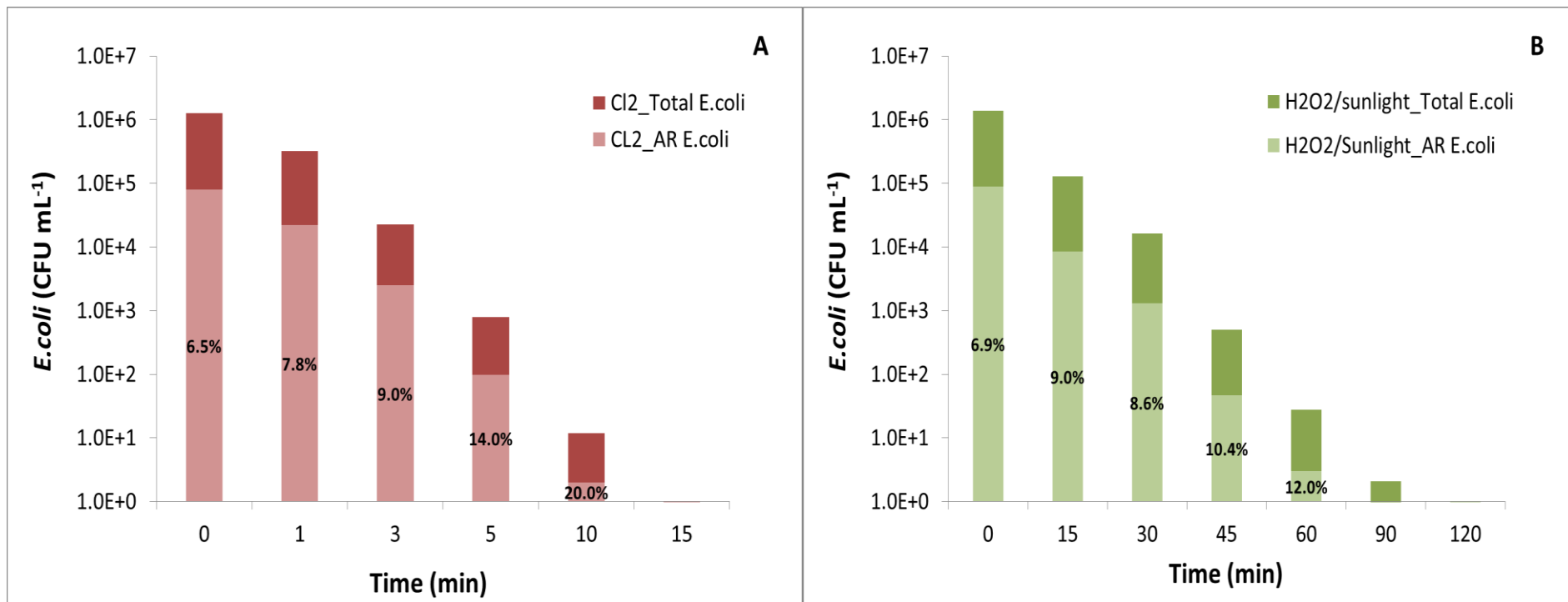
Effect of photo driven AOPs on ARB

- Effect of H₂O₂/sunlight (a), TiO₂/sunlight and TiO₂/H₂O₂/sunlight (b) processes on multi-drug resistant *E. coli* strain (ampicillin, ciprofloxacin, tetracycline)



Effect of photo driven AOPs on ARB

- Comparison between Cl_2 (1 mg/L, A) and sunlight/ H_2O_2 (50 mg/L, B)



Effect of conventional disinfection on ARGs

■ Lab scale evidences

- ✓ **UV-C** doses ranging from 200 to 400 mJ cm⁻² (at least one order of magnitude higher than those for the inactivation of host bacterial cells and impractical for water utilities) were required to remove 3 or 4 Log units of ARGs, namely *ampC*, *mecA*, *tetA* and *vanA* (McKinney and Pruden, 2012);
- ✓ Lower **UV-C** doses (5 -10 mJ cm⁻²) were found to be effective in the removal of *ereA*, *ereB*, *ermA*, *ermB*, *tetA*, *tetO* starting from lower initial ARGs copies mL⁻¹ (Guo et al., 2013);
- ✓ ARGs *ereA* and *ermB* persisted in urban wastewater **chlorinated** (15 mg Cl₂ min L⁻¹) samples (Yuan et al. 2015) ...
- ✓ ... and **chlorination** was found to be effective in ARGs removal (3.16 Log for *sulI* and 3.24 Log for *tetG* after 120 min treatment) only at non-realistic chlorine concentration (160 mg L⁻¹) (Zhuang et al., 2015). **Ozonation** (177.6 mg L⁻¹) also decreased ARGs (1.68–2.55 log) but the relative abundance of the selected genes increased during ozonation and with low doses of UV and chlorine.

Effect of conventional disinfection on ARGs

■ Evidences from full scale UWWTPs

- ✓ Overall disinfection by chlorination and UV radiation (5 UWWTPs) did not prove to have significant contribution to ARGs (tetw, tetO, sull) and ARB reduction (Munir et al., 2011).
- ✓ The Statistical t-test between concentrations of ARGs abundance in pre- and post disinfected effluent did not show any significant difference (t-test, $p > 0.05$) between UV and chlorination disinfection processes (Munir et al., 2011).
- ✓ Higher removal (1-3 Log units) of tetW gene was observed in samples after MBR and UV radiation processes, while other ARGs were still present in the final effluents (Munir et al., 2011).
- ✓ No significant differences ($p > 0.05$) in tet and sul genes were observed before and after chlorination in an UWWTP (Gao et al., 2012).

Effect of photo driven AOPs on ARGs

- Effect of sunlight/H₂O₂ process on selected AR *E. coli* strain and ARGs (intracellular and total DNA) in aqueous suspension

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β -lactams resistance gene quantification in an antibiotic resistant *Escherichia coli* water suspension treated by advanced oxidation with UV/H₂O₂



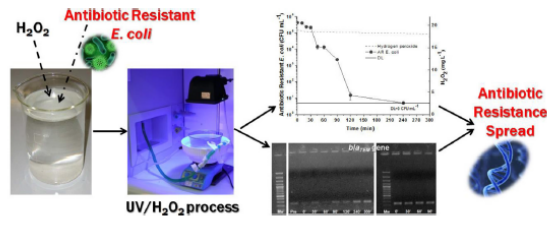
Giovanna Ferro^a, Francesco Guarino^b, Angela Cicatelli^b, Luigi Rizzo^{a,*}

^a Department of Civil Engineering, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, SA, Italy
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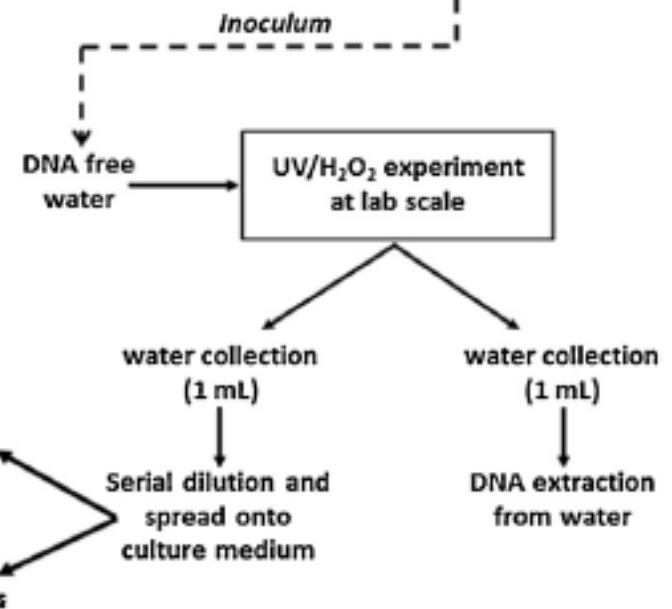
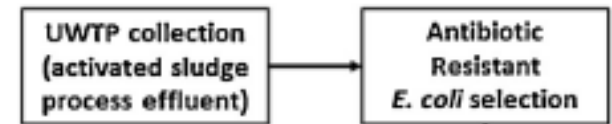
HIGHLIGHTS

- An antibiotic resistant *E. coli* strain has been effectively inactivated by UV/H₂O₂ process.
- Bacterial cells released antibiotic resistant genes (ARGs) during UV/H₂O₂ treatment.
- UV/H₂O₂ process did not affect antibiotic resistance of survived colonies.
- UV/H₂O₂ process did not significantly change the copy number per mL of blaTEM gene.

GRAPHICAL ABSTRACT



➤ Selection of Antibiotic Resistant *E. coli* strain

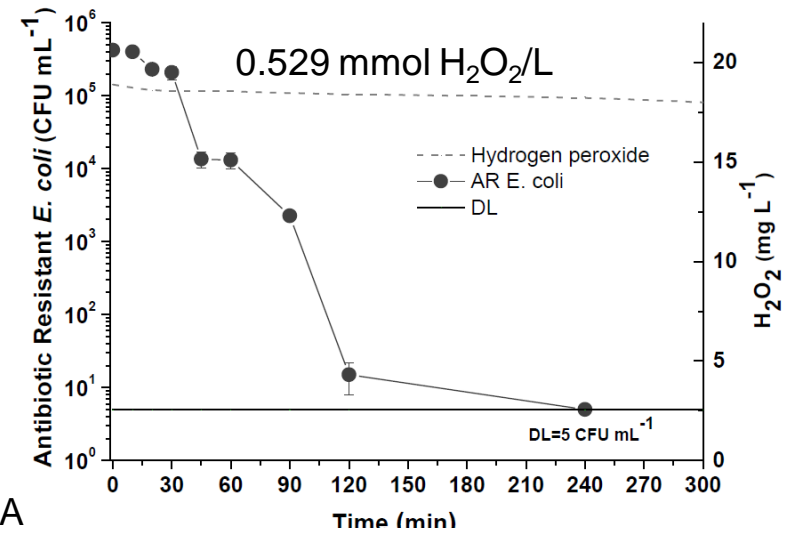


➤ DNA extraction

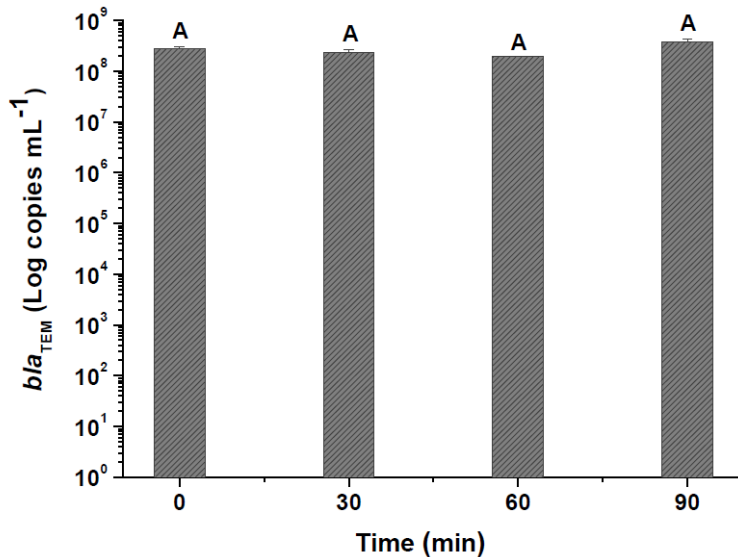
Fig. 1. Schematic view of experimental design.

Effect of photo driven AOPs on ARGs

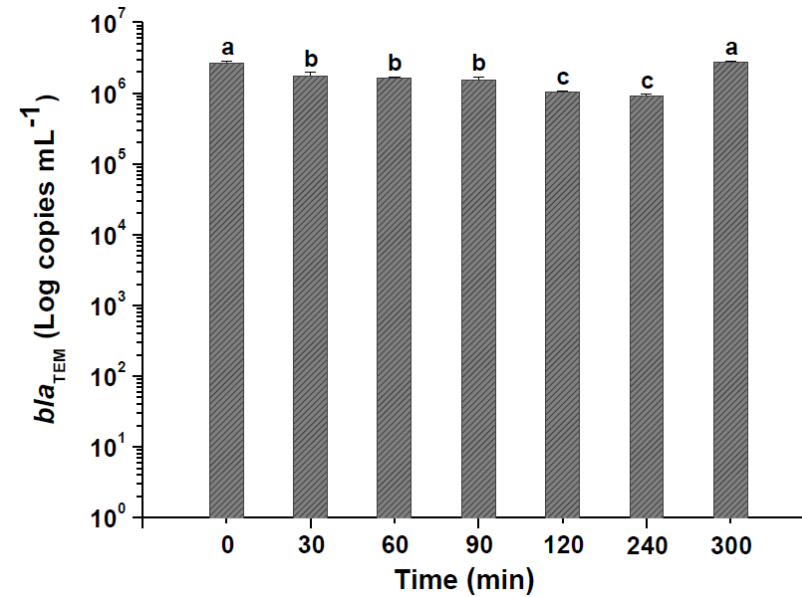
- Effect of sunlight/H₂O₂ process on selected AR *E. coli* strain and ARGs (intracellular and total DNA) in aqueous suspension



Intracellular DNA



Total DNA



Effect of photo driven AOPs on ARGs

- Effect of sunlight/H₂O₂ process on indigenous AR *E. coli** and ARGs (in intracellular and total DNA) in urban WW.

Science of the Total Environment 560-561 (2016) 29-35



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Science of the Total Environment

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



Antibiotic resistance spread potential in urban wastewater effluents disinfected by UV/H₂O₂ process



Giovanna Ferro ^a, Francesco Guarino ^b, Stefano Castiglione ^b, Luigi Rizzo ^{a,*}

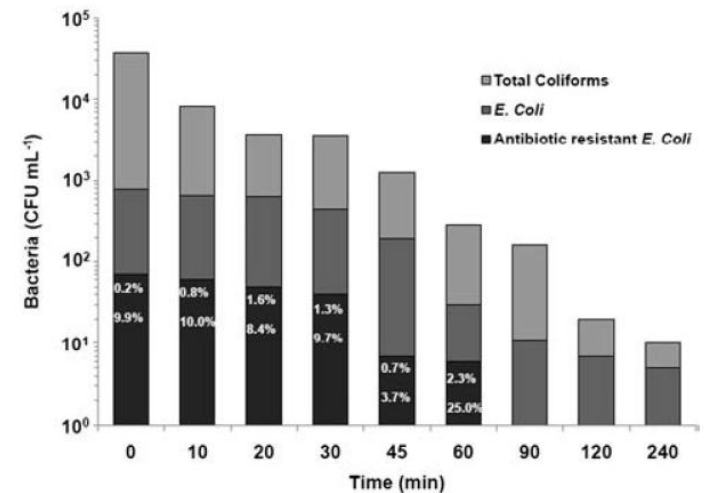
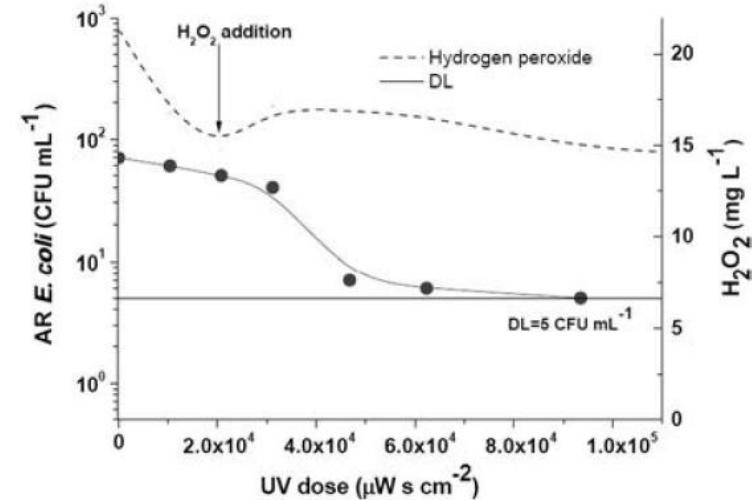
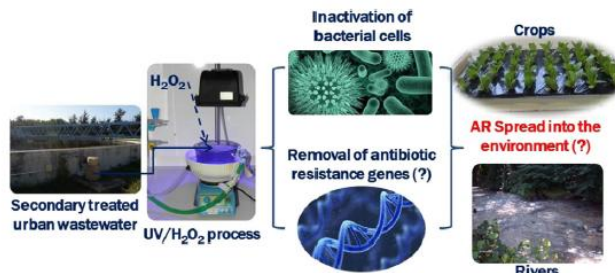
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HIGHLIGHTS

- Bacterial inactivation and ARGs fate were simultaneously investigated.
- Antibiotic resistant *E. coli* were effectively inactivated in real wastewater.
- A decrease of *bla*_{TX4} and *qnrS* genes in intracellular DNA was observed.
- UV/H₂O₂ process was not effective in ARGs removal from wastewater.
- DNA release in the treated water may contribute to antibiotic resistance spread.

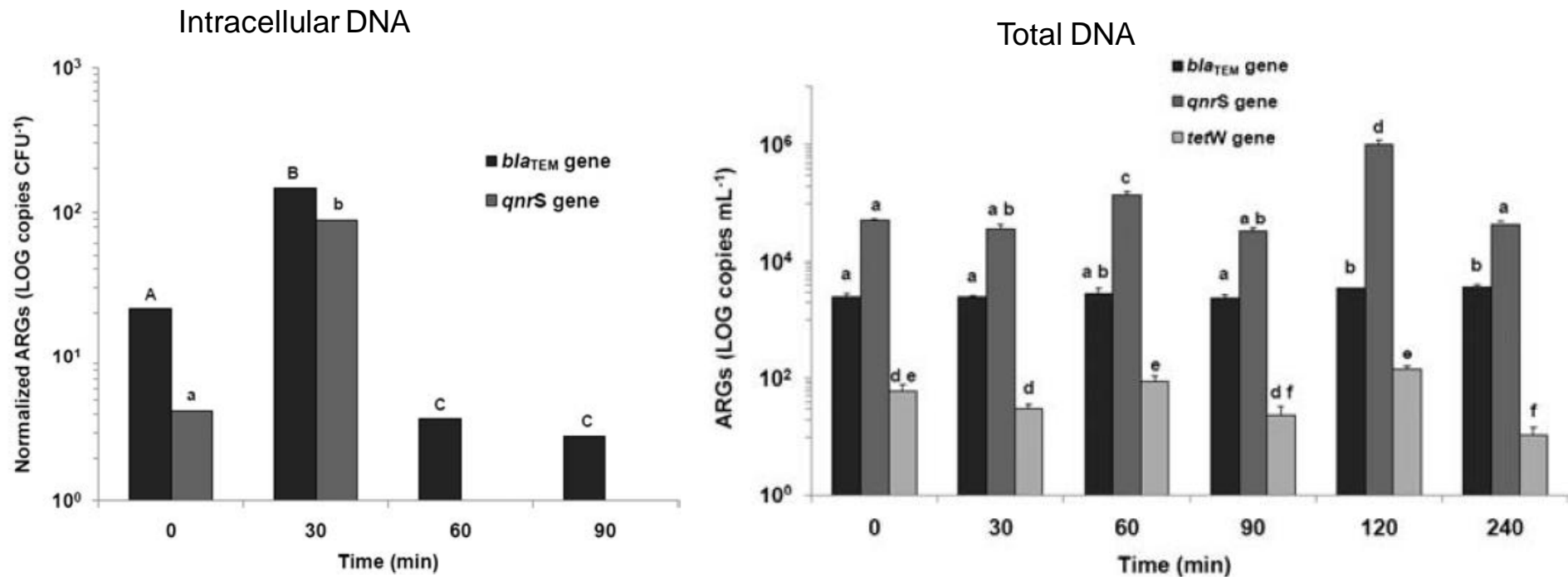
GRAPHICAL ABSTRACT



*8 mg/L of ampicillin, 0.064 mg/L of ciprofloxacin, 8 mg/L of tetracycline

Effect of photo driven AOPs on ARGs

- Effect of sunlight/H₂O₂ process on indigenous AR *E. coli* and ARGs (in intracellular and total DNA) in urban ww.



(Ferro et al., 2016, Science of the Total Environment 560-561, 29-35)

Effect of photo driven AOPs on ARGs

- Effect of UV-C/H₂O₂ process on ARGs in urban ww compared to Fenton.

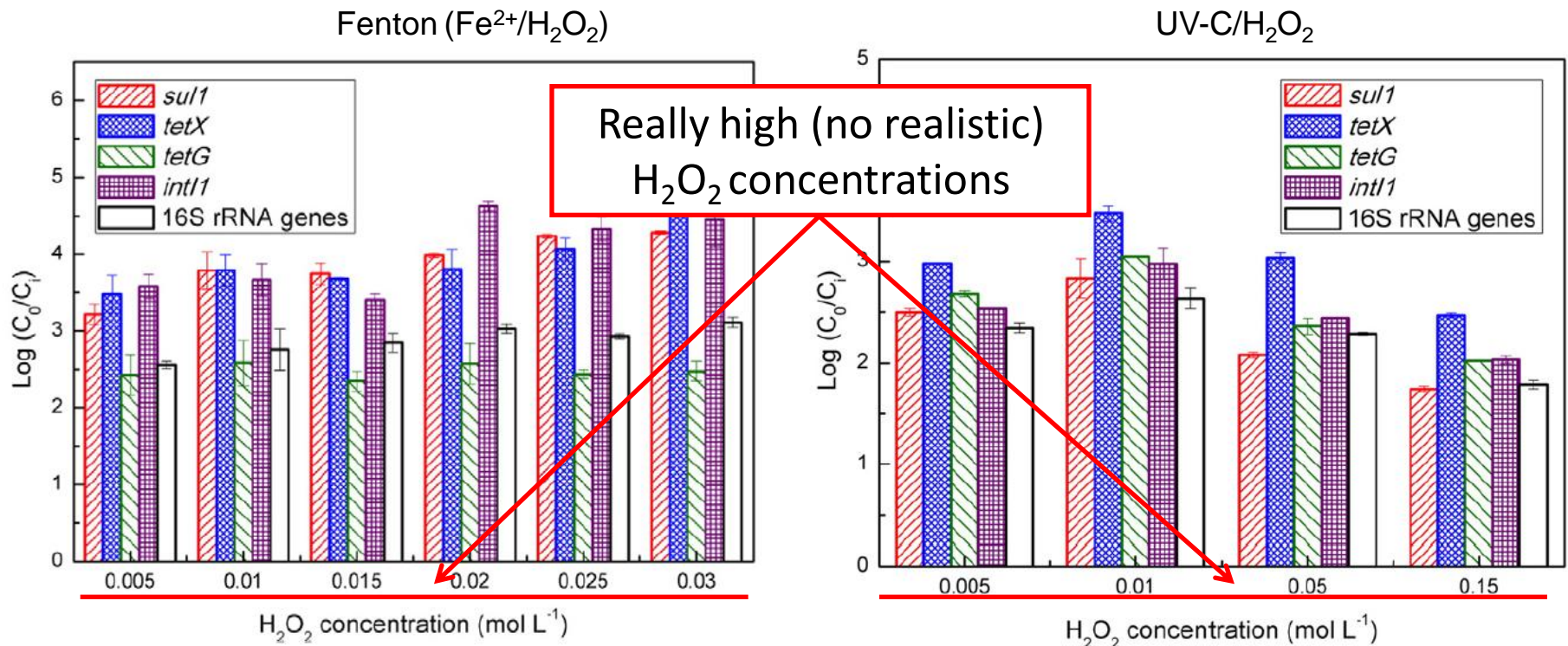
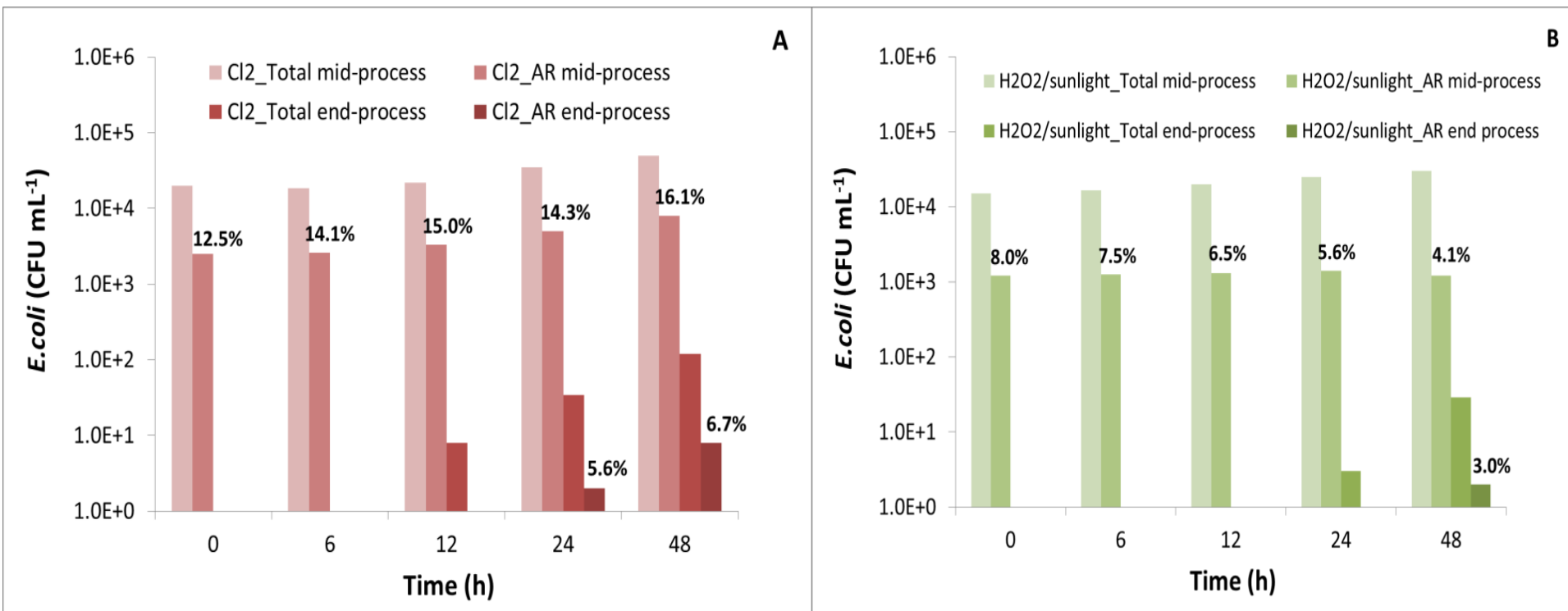


Fig. 2. Effect of H₂O₂ concentration on reduction of target genes (Fe²⁺/H₂O₂ molar ratio = 0.1, initial solution pH = 3.0, reaction time = 2 h).

Fig. 6. Effect of H₂O₂ concentration on reduction of target genes (UV irradiation time = 30 min, initial solution pH = 3.5).

Wastewater disinfection by AOPs: ARB regrowth

- Total and AR *E. Coli* regrowth: comparison between solar/H₂O₂ (50 mg/L) and chlorination (Cl₂, 1 mg/L)



Conclusions

- Although conventional and new disinfection processes can be effective in the inactivation of ARB, they may be not effective in controlling AR spread. In particular:
 - ✓ Conventional disinfection processes (namely, chlorination, UV-C radiation and ozonation) can eventually remove ARGs ... but under not realistic operative conditions (in terms of oxidant/disinfectant concentration, UV radiation);
 - ✓ A few investigations at full scale showed that chlorine and UV-C disinfection do not significantly affect ARGs abundance;
 - ✓ Only a few works investigated the effect of AOPs on ARGs and results depend on target AOP and ARGs.
- Further studies need to understand:
 - ✓ The effect of AOPs on the potential of AR transfer (ARB&Gs and mobile genetic elements release in the effluent).
 - ✓ If potential AR transfer can actually result into a real AR transfer.

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