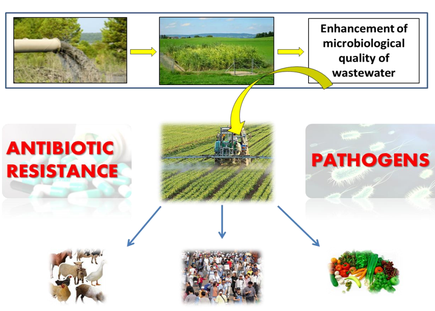
REMOVAL OF ANTIBIOTIC RESISTANCE GENES BY TWO INNOVATIVE WASTEWATER TREATMENT SOLUTIONS FOR WATER REUSE IN AGRICULTURE

M.L. Luprano[1], C.Levantesi[1], M. De Sanctis[2], C. Di Iaconi[2], A. Toscano[3], A. Marzo[3]

[1] Water Research Institute, CNR, Via Salaria Km 29.600, 00015 Monterotondo (RM), Italy
[2] Water Research Institute, CNR, Via F. De Blasio 5, 70123 Bari, Italy
3 Department of Agricultural, Food and Environmental, University of Catania, Catania, Italy

BACKGROUND



- Treated-wastewater (TWW) irrigation is becoming increasingly prevalent in arid regions of the world, due to growing demand and decline in freshwater supplies
- Detrimental microbial effects associated with reclaimed water may hinder the use of recycled water for agricultural irrigation. In particular, the spreading of ARGs into the environment has recently raised a great concern for the risk of transferring antibiotic resistance to pathogens
- Wastewater treatment plants (WWTPs) have been described to possess the ideal mix of conditions to promote horizontal gene transfer and development of antibiotic resistant bacteria

OBJECTIVES

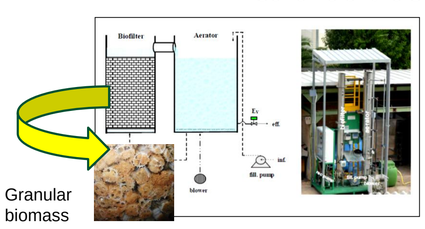
AIM: To evaluate the **fate and removal of antibiotic resistance genes (ARGs)** in two different wastewater treatment solutions for **water reuse in agriculture**:

- a technological treatment solution comprising enhanced secondary treatment by SBBGR (*Sequencing Batch Biofilter Granular Reactor*) at pilot- scale, combined with UV disinfection
- a natural wastewater treatment system made up of an horizontal subsurface flow constructed wetland (CW) at full scale, followed by lagooning

MATERIALS AND METHODS

Technological treatment solution

Scheme of the SBBGR technology



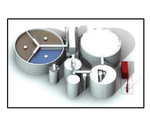
UV disinfection

SBBGR pilot-scale
- High biomass concentration (30-50 kg/m^3)
- Volume: 330 L
- HRT: ≥ 200 d

Sampling points
- Raw influent
- SBBGR effluent
- Sand-filter treated effluent
- UV-treated effluent

Natural treatment solution

Scheme of the Constructed Wetland



Municipal wastewater treatment plant

H-SSF CW

lagooning

H-SSF Constructed wetland
- ~ 2,000 m2 in operation since 2012
- 8–15 mm volcanic gravel (porosity 0.47, depth 0.6 m)
- Phragmites australis (Poaceae Fam.)
- Flow rate 2 L/s

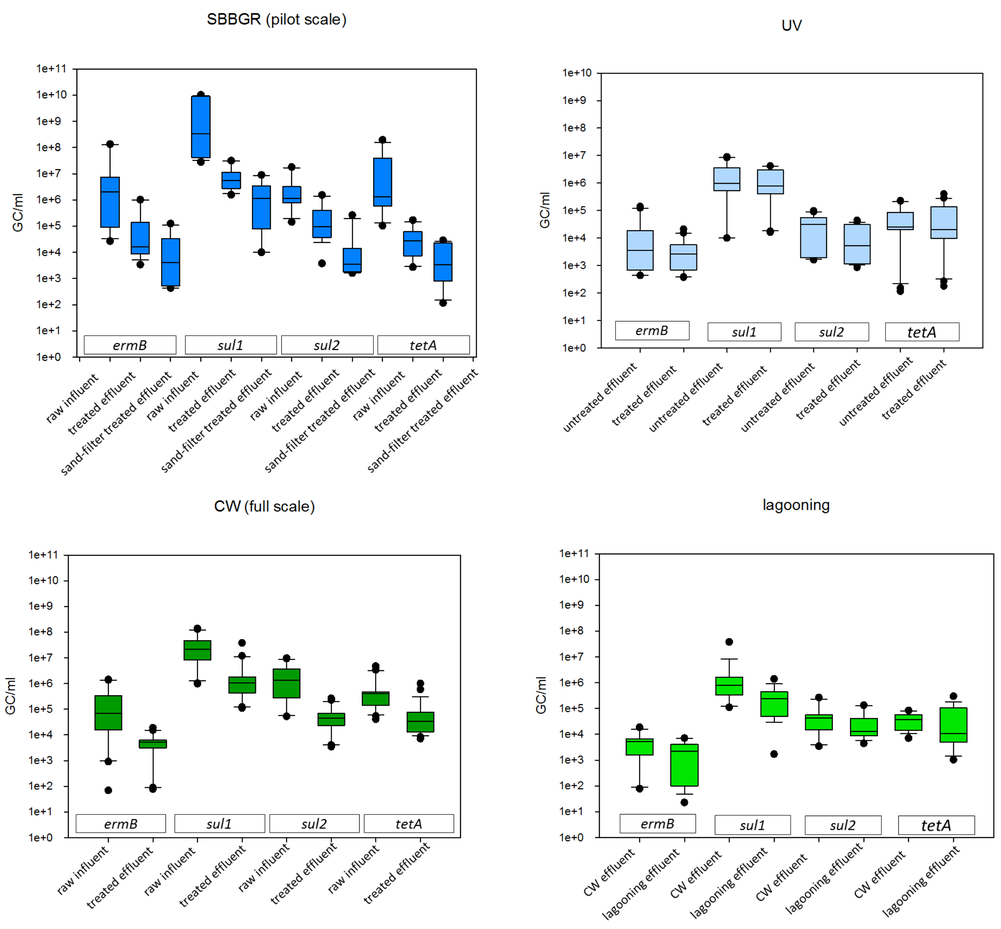
Samplign points
- Raw influent
- CW effluent
- Lagooning effluent

Sampling campaigns:
6 for the SBBGR pilot scale
10 for the Constructed Wetland

Biomolecular tools:
- PCR screening
- qPCR quantification

RESULTS AND DISCUSSION

ARG level

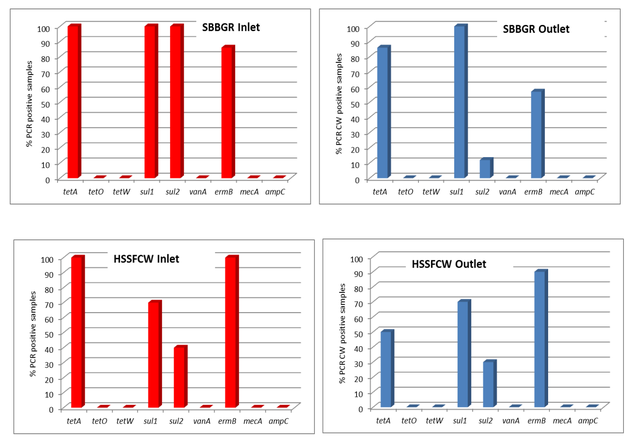


- ✓ Q-PCR quantification revealed abundant level of all the ARGs in the raw wastewater ranging from 6.6 x 10^2 of *ermB* to 9.6 x 10^9 of *sul1*
- ✓ ARG level in the secondary effluent ranged from 8.7 x 10^1 of *ermB* to 3.7 x 10^7 of *sul1*
- ✓ Statistically significant reduction (*t* test, *p-value*<0.05) was observed for all the genes on a volumetric basis by both SBBGR and CW treatments
- ✓ no significant (*t* test, *p-value*>0.05) was observed for the tested ARGs before and after UV
- ✓ Statistically significant reduction (*t* test, *p-value*<0.05) was observed for three genes *ermB*, *sul1* and *sul2* after lagooning

ARG diversity

- ✓ 9 ARGs commonly found in WWTPs (see table) and encoding resistance to 5 of classes of antibiotics, were selected and their presence in 16 samples of raw influent and treated effluent was analyzed by PCR screening
- ✓ 4 of the analyzed ARGs, namely ***ermB***, ***sul1***, ***sul2*** and ***tetA***, were detected by PCR
- ✓ Surprisingly, **limited diversity** was observed, the same ARGs were in fact detected in the two systems in spite of the different influent characteristics and the diverse geographical locations

TETRACYCLINES (*tetA, tetW, tetO*)
SULFONAMIDES (*sul1, sul2*)
GLYCOPEPTIDES (*vanA*)
MACROLIDES (*ermB*)
BETA-LACTAM (*ampC, mecA*)



ARG removal

Table 1. ARGs average logarithmic removals achieved by technological (SBBGR) treatment solution

Target ARGs	SBBGR	Sand filter	UV
	Log$_{10}$ removal (GC/ml)		
ermB	1.67 ± 0.70	1.01 ± 0.80	0.42
sul1	1.89 ± 0.80	1.02 ± 0.90	0.29
sul2	1.08 ± 0.45	1.24 ± 0.83	0.26 ± 0.24
tetA	2.08 ± 1.13	0.82 ± 0.74	0.26 ± 0.28

Table 2. ARGs average logarithmic removals achieved by natural (CW) treatment solution

Target ARGs	CW	lagooning
	Log$_{10}$ removal (GC/ml)	
ermB	1.51 ± 0.71	1.02 ± 0.67
sul1	1.26 ± 0.87	0.67 ± 0.54
sul2	1.46 ± 0.78	0.49 ± 0.27
tetA	1.19 ± 0.55	0.70 ± 0.02

- ✓ The SBBGR treatment efficiently reduced the level of all the ARGs during the whole monitored period, achieving log reduction ranging from 1.08 ± 0.45 log units of GC/ml of *sul2* to 2.08 ± 1.13 log units of GC/ml of *tetA.* SBBGR system up-grated with sand filtration improved the effluent quality in terms of ARG reduction
- ✓ Similarly, the CW system reduced ARGs level although with variable efficiencies between genes and during the different sampling seasons (highest ARG reduction observed in the summer period (data not shown)
- ✓ No significant ARG removal (< 1 log unit of GC/ml) was always obtained by UV
- ✓ Lagooning sligtly improoved the effluent quality in terms of ARGs reduction

CONCLUSIONS

- ➢ The observed ARG reduction was mainly due to the applied secondary treatment (SBBGR and CW)
- ➢ No or slight additional improvement of the effluent quality in terms of ARGs presence is obtained by tertiary treatments, namely UV and lagooning, with the exception of the enhancement based on the filtration step in the SBBGR system
- ➢ ARGs are still present in the final effluent for agricultural reuse (**any risk associated to be assessed**??)

References:
- Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Science of the Total Environment 2013; 447:345-360.
- Fahrenfeld N, Ma Y, O'Brien M, Pruden A. Reclaimed water as a reservoir of antibiotic resistance genes: distribution system and irrigation implications. Frontiers in Microbiology 2013; 4:130