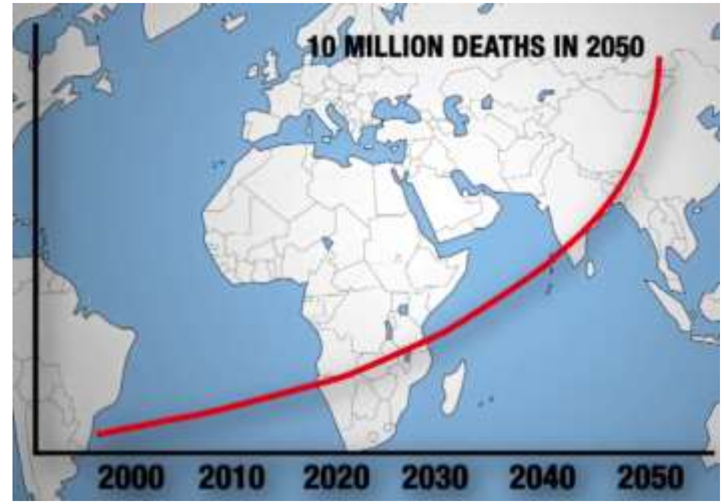
Fate of antibiotic resistance during advanced waste water treatment

Soňa Fajnorova, Uwe Hübner, Bastian Herzog, Johann Müller,
Karin Hellauer, David Miklos, Jörg E. Drewes, Jiří Wanner

Vodárenská biologie 6-7 Februar 2018

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE
TECHNISCHE UNIVERSITÄT MÜNCHEN
Bayerisches Staatsministerium der Finanzen, für Landesentwicklung und Heimat

Antibiotic resistance – most serious threat of 21st century



Médecins Sans Frontières (MSF, 2017)

Hampton T. (2013)

World Health Organization

Media centre | Publications | Countries | Programmes | Governance

Essential medicines and health products

Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics

Publication details

Number of pages: 7
Publication date: 27 February 2017
Languages: English

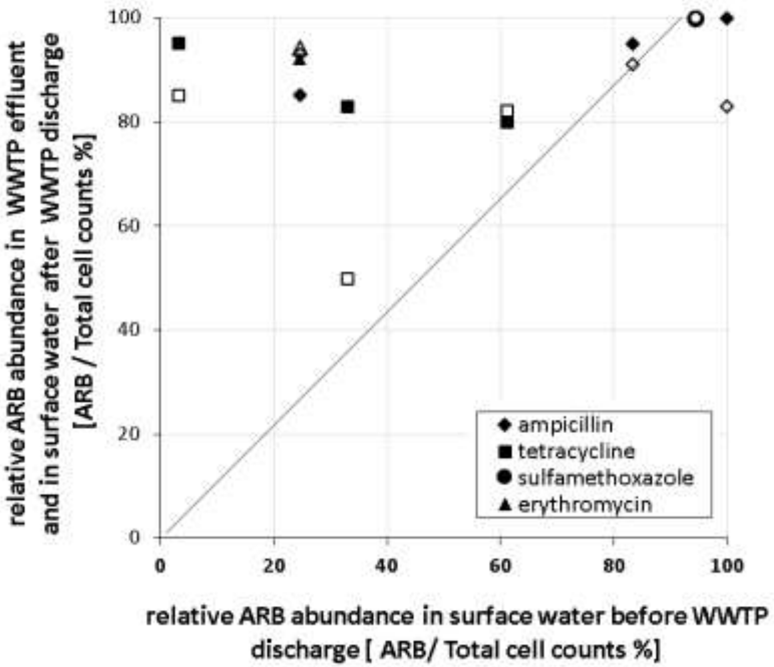
Geographical distribution of ARGs in water bodies

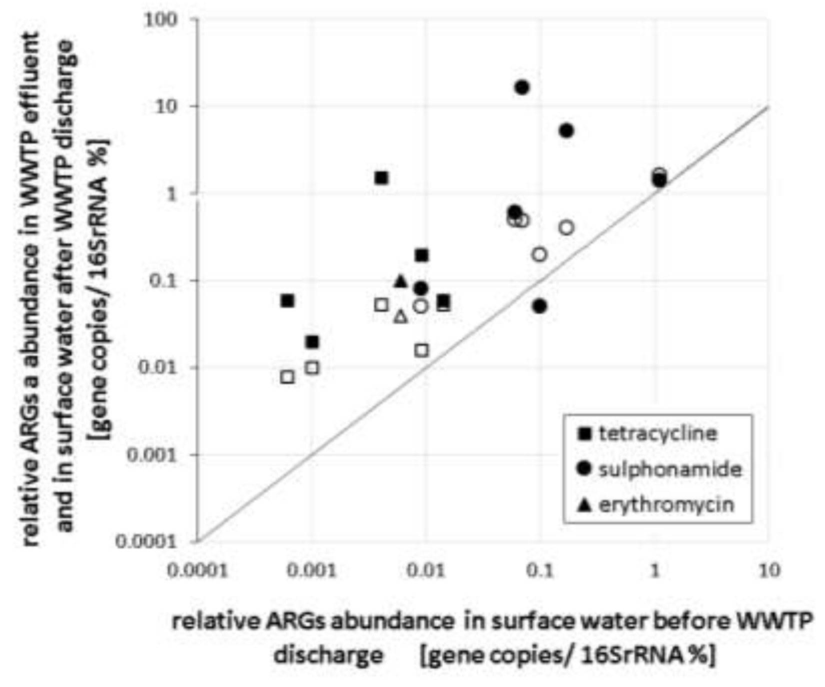
Genes encoding resistance to ◆sulphonamide, ○ tetracycline, + β-lactam, ▲ macrolide, ★ trimethoprim , ■ aminoglycoside and ▼ chloramphenicol

Zhang X et al. (2008)

WWTP - critical point sources of antibiotic resistance

- > 80% of antibiotic resistant bacteria in WWTP effluent
- ↑ of antibiotic resistance in surface water after the discharge of WWTP





Full symbols - WWTP effluents, Empty symbols - surface water after WWTP discharge

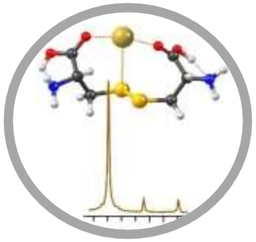
Hiller et al. (in preparation)

Water quality regulations and new challenges

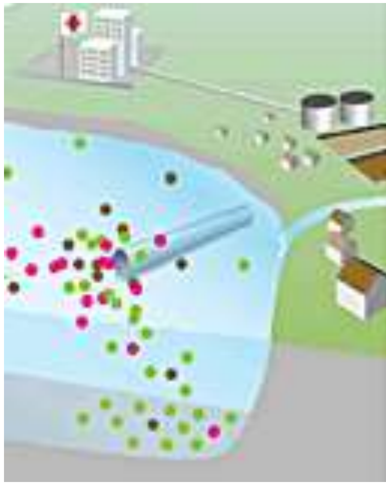
- **Microbial parameters**
  Public health assessment

Depositphotos.com

- **Trace organic chemicals (TOrCs)**
  Environmental risk assessment



Earthsciences.hku.hk



Eawag.ch

ADVANCED WASTEWATER TREATMENT

?

- **Antibiotic resistance (ARB & ARGs)**
  Public health and environmental risk assessment

Simplebiologyy.blogspot.de

AMR – Advanced wastewater treatment

The objective of this study was to assess and compare the fate and transport of antibiotic resistance in selected advanced wastewater treatment processes:

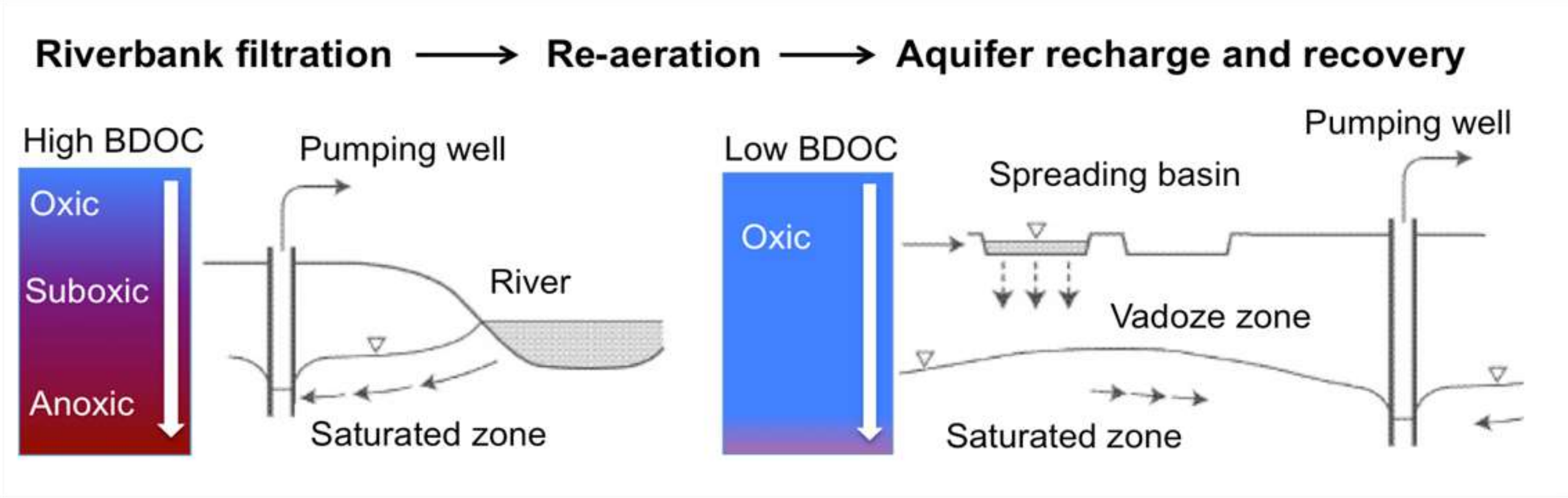
- UV disinfection and AOP (UV/H_2O_2)
- Ozonation
- Sequential Biofiltration
- Sequential Managed Aquifer Recharge Technology

Managed Aquifer Recharge (MAR)

RBF, SAT, AAR:efficient barrier for many contaminants

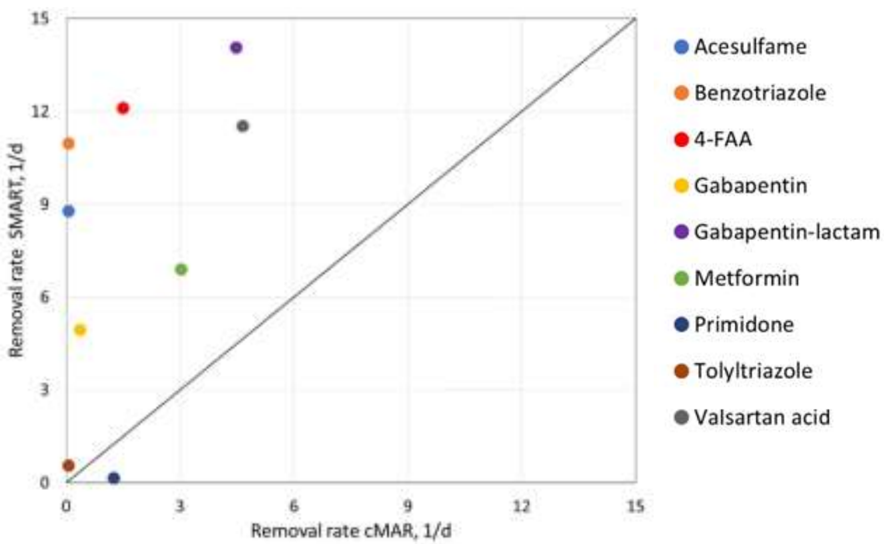
Oxic and **carbon limited conditions** are favorable for an **enhanced TOrCs removal**

Sequential Managed Aquifer Recharge Technology (SMART)

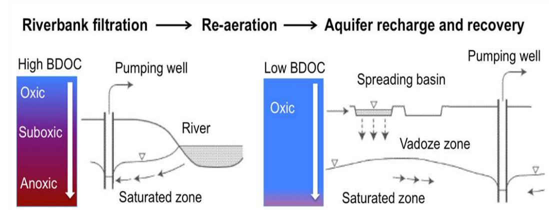


Regnery et al. (2016)

Sequential Managed Aquifer Recharge Technology (SMART)



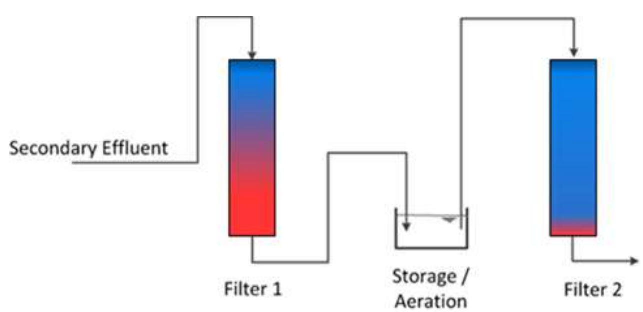
Hellauer et al. (2017)



Regnery et al. (2016)

- Oxic and carbon limited conditions
- Enhanced TOrCs removal
- Full scale: Aurora (CO), Lake Tegel (Berlin)

Sequential Biofiltration (SBF)



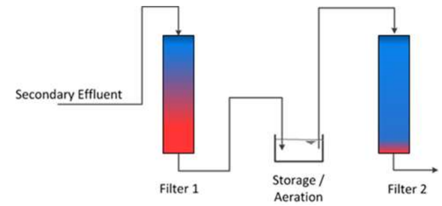
Müller et al. (2017)

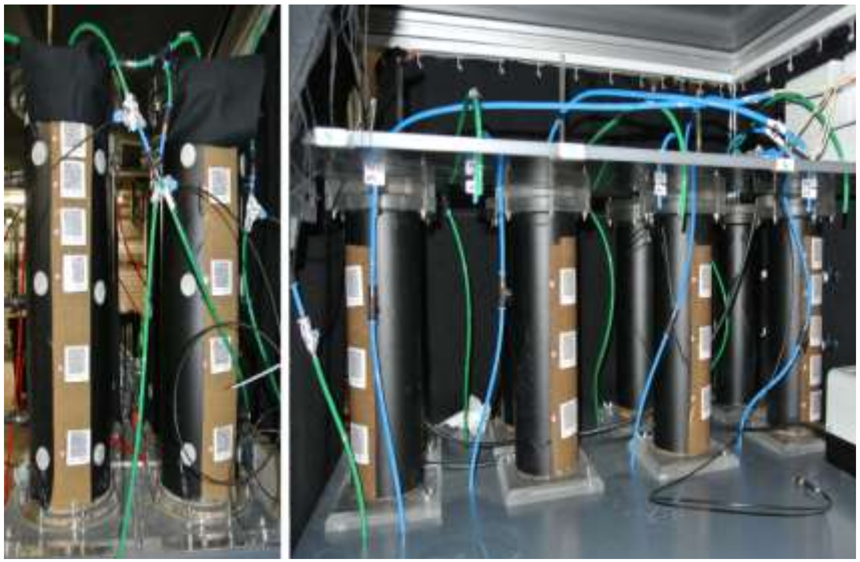
- Oxic and carbon limited conditions
- Enhanced TOrCs removal
- Reduction of hydraulic retention time

Experimental Set-up

- TUM research centre: lab and pilot-scale facilities
- WWTP Garching: fresh secondary effluent

Experimental Set-up





SMART

SBF

- Infiltration stage:
  1st: Technical sand, HRT = 4.2 d
  2nd: Aquifer material, HTR = 3.6 d

- Infiltration stage:
  1st: Anthracite, EBCT = 90 min
  2nd: Technical sand, EBCT = 200 min

Experimental Set-up

UV disinfection and AOP

- UV disinfection: 500 - 16,000 J/m^2
- AOP: 500 - 16,000 J/m^2,9.5±0.3 mg H_2O_2/L

Ozone – semi batch reactor

- Up to 15 mg O_3/L (0.76 mg O_3/mg DOC)

Target microbial indicator and ARGs

Priority class of ARB	Bacteria	ARGs	Drug	Antibiotic class
Critical	***P. aeruginosa***	**blaVIM**	Imipenem	last resort
	Enterobacteriace	**ampC**	Ampicilin	broad spectrum
	E.coli	**sul1**	Sulfamethoxazole	
High	***Enterococci***	**van A**	Vanomycin	last resort
Medium	*Streptococcus pneumoniae*	**ermB**	Erythromycin	broad spectrum

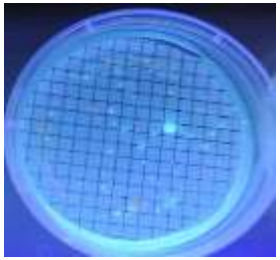
- **16S rRNA** - total cell concentration

Determination of FIB and ARGs

- **Culture based methods**

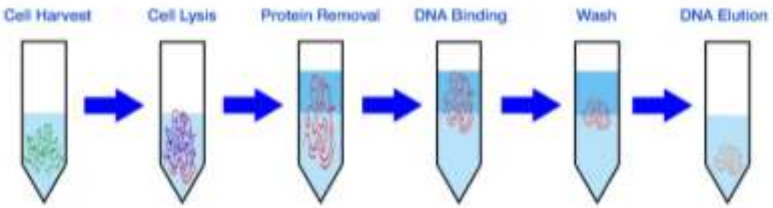
Escherichia coli
Total Coliform bacteria
ISO 9308-1:2014 2014

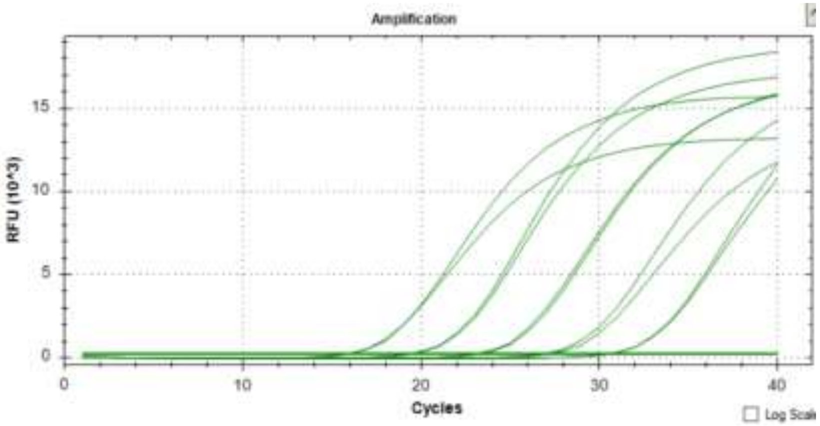
Intestinal Enterococci
ISO 7899-2:2000



Pseudomonas aeruginosa
ISO 16266:2006

- **Molecular based methods (qPCR)**





Quality parameters of the secondary effluent

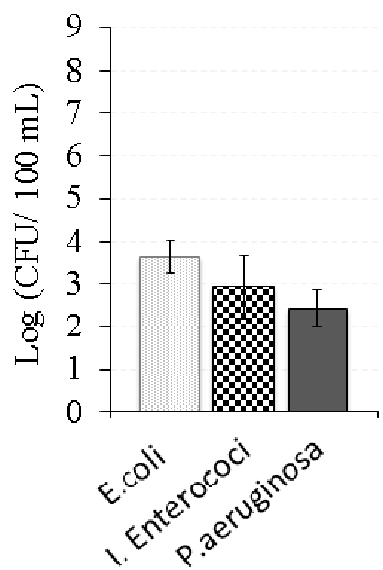
- WWTP Garching (31,000 PE)

Bulk parameters

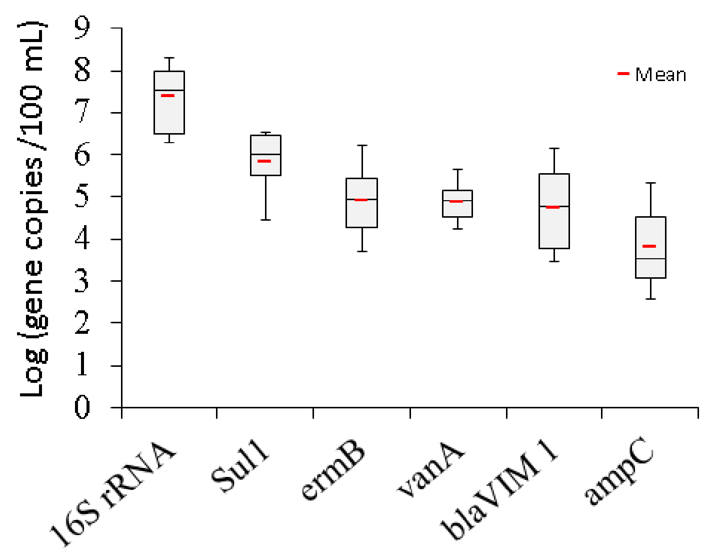
Parameter	Unit	Value
T	°C	19.00 ± 1.13
pH	-	7.79 ± 0.15
conductivity	µS/cm	1187 ±190
NH_4-N	mg/L	0.90 ± 1.07
NO_2-N	mg/L	0.51 ± 0.31
NO_3-N	mg/L	23.60 ±5.71
UV_{254}	A/m	17.80 ± 1.95
$SUVA_{254}$	L/(mg.m)	1.58 ± 0.46
DOC	mg/L	9.38 ± 2.34
TOC	mg/L	11.10 ± 2.04

n=15

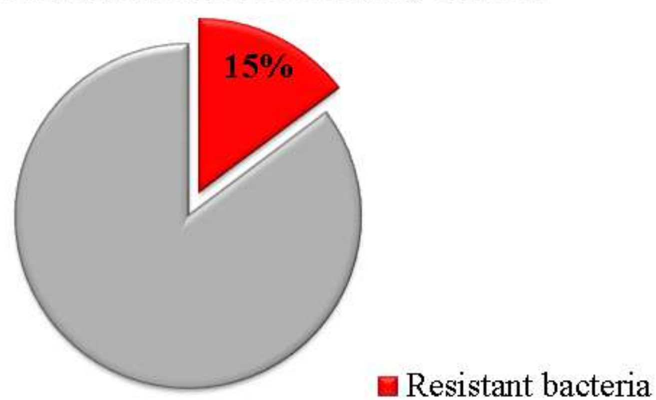
Abundance of FIB



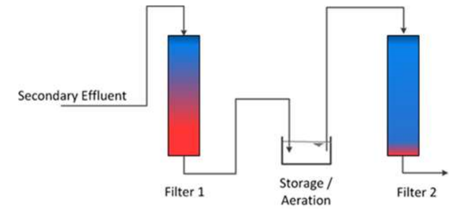
Abundance of 16SrDNA and ARGs



Ratio of resistant bacteria in secondary effluent

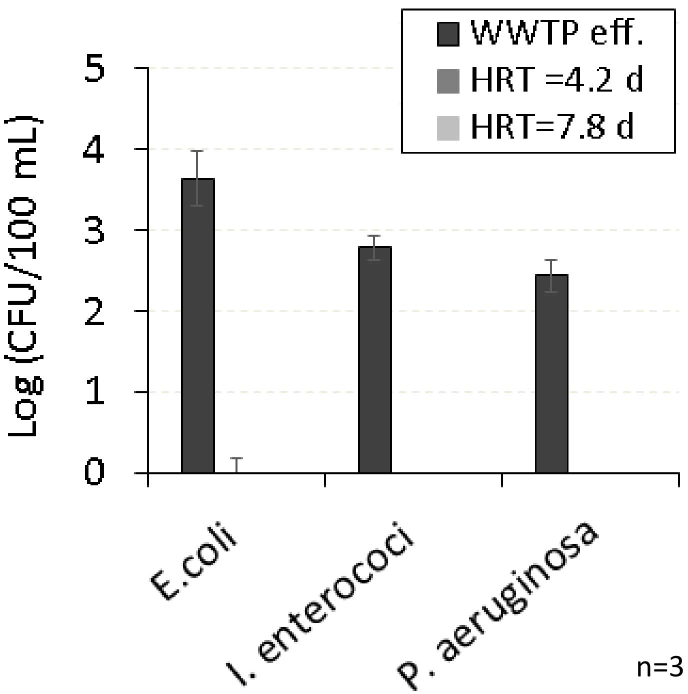


Performance of SMART

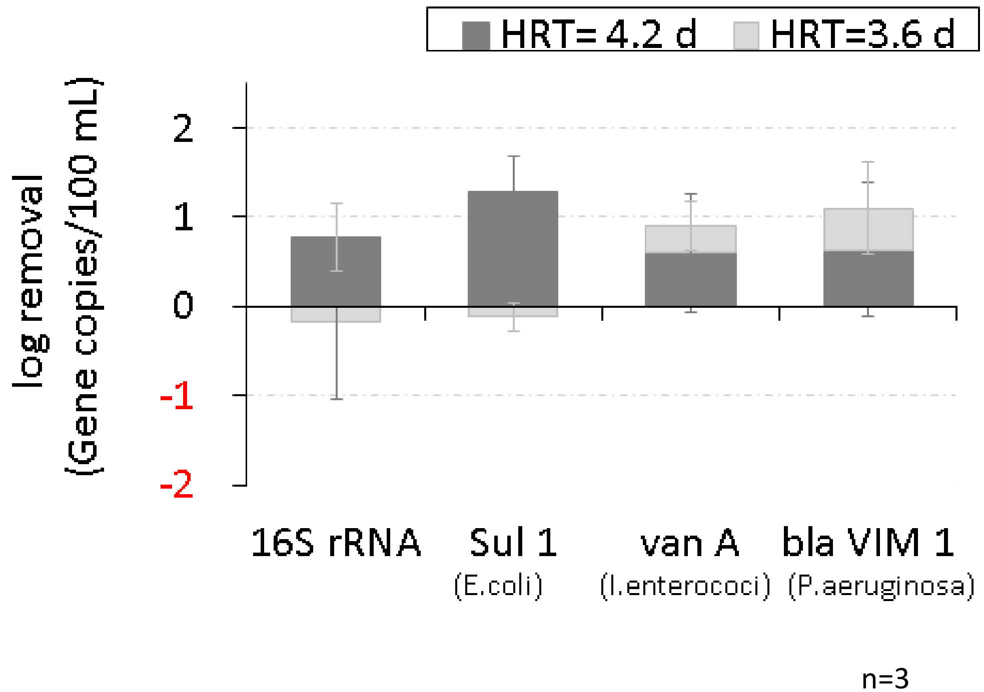


HRT=4.2d HRT=3.6d

Abundance of FIB before and after treatment

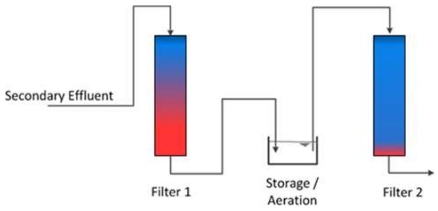


Elimination of target genes



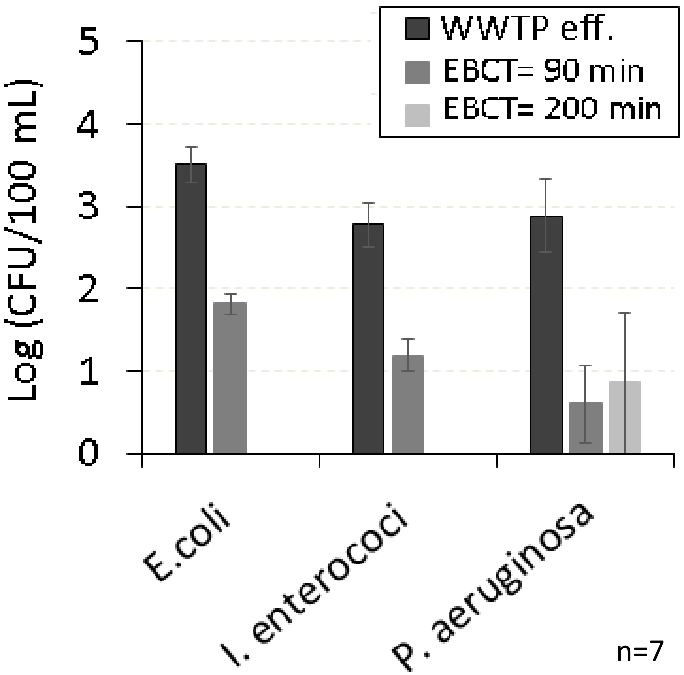
- Complete inactivation of FIB in the 1st infiltration stage
- Major gene removal in the 1st infiltration stage
- Enhanced removal of vanA and BlaVIM genes in the 2nd infiltration stage

Performance of SBF



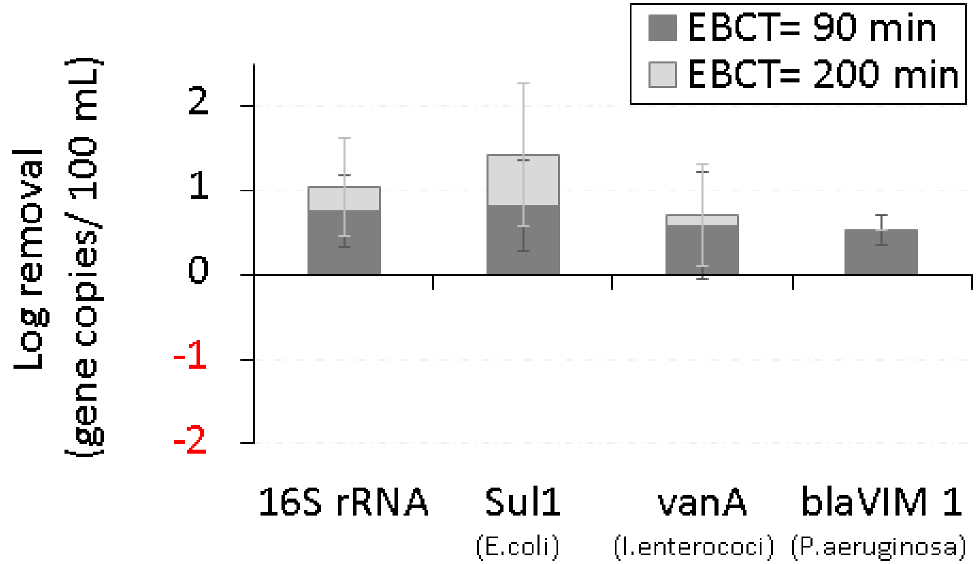
EBCT=90min EBCT=200min

Abundance of FIB before and after treatment



n=7

Elimination of target genes

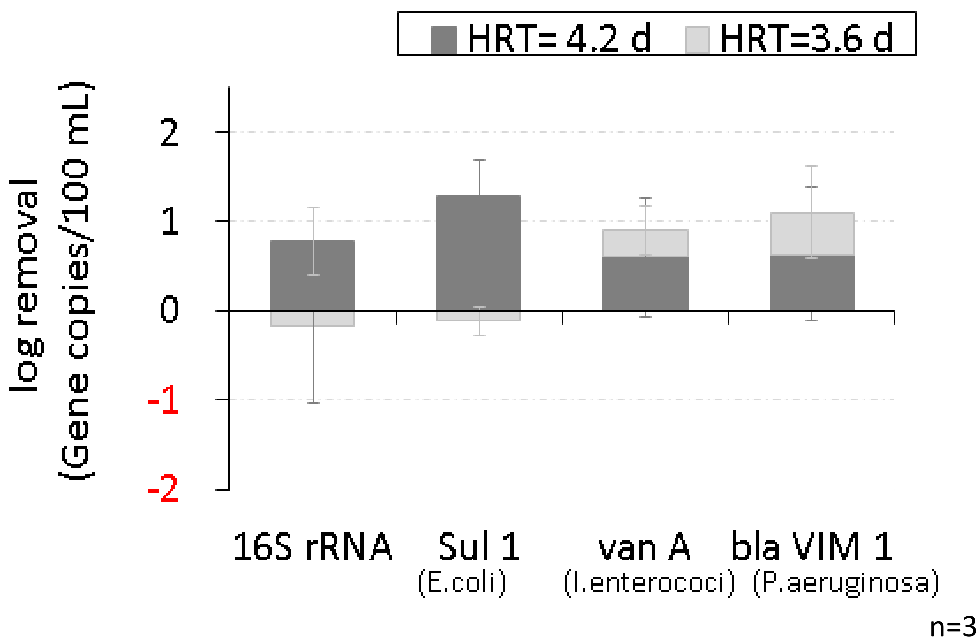


n=5

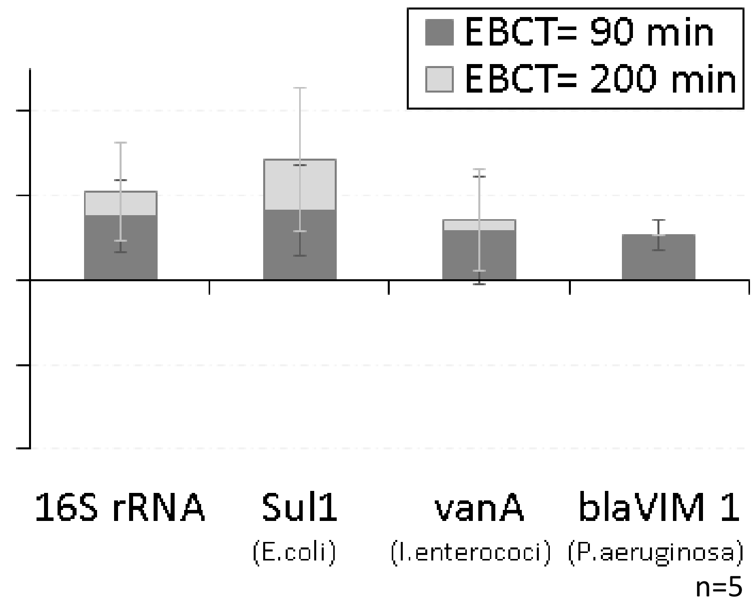
- Complete inactivation of E.coli and i. Enterococci, 2.2 log reduction P. aeruginosa
- Major gene removal in the 1st infiltration stage
- Enhanced removal of 16SrRNA and Sul1 genes in the 2nd infiltration stage

Elimination of target genes in SMART and SBF

SMART



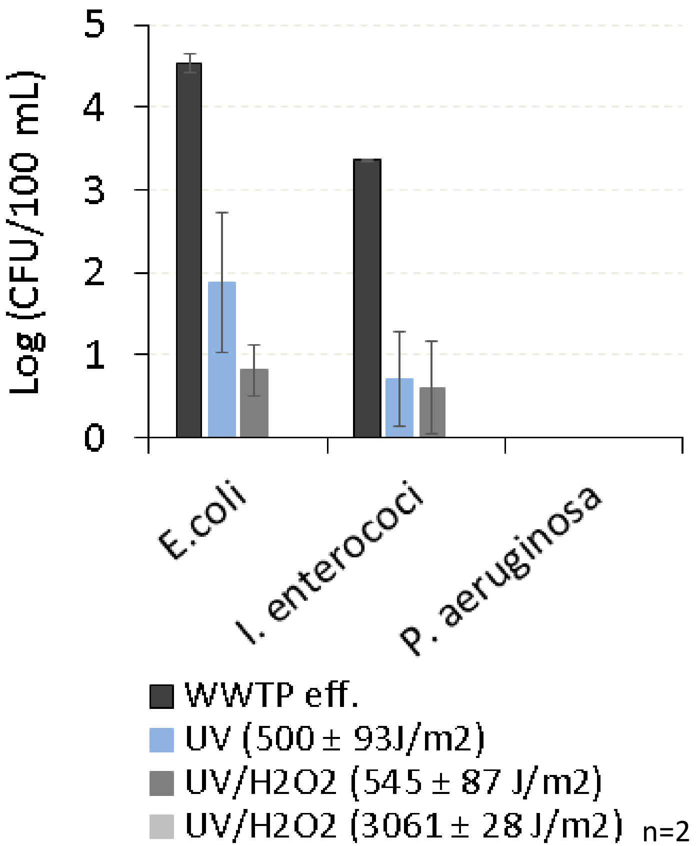
SBF



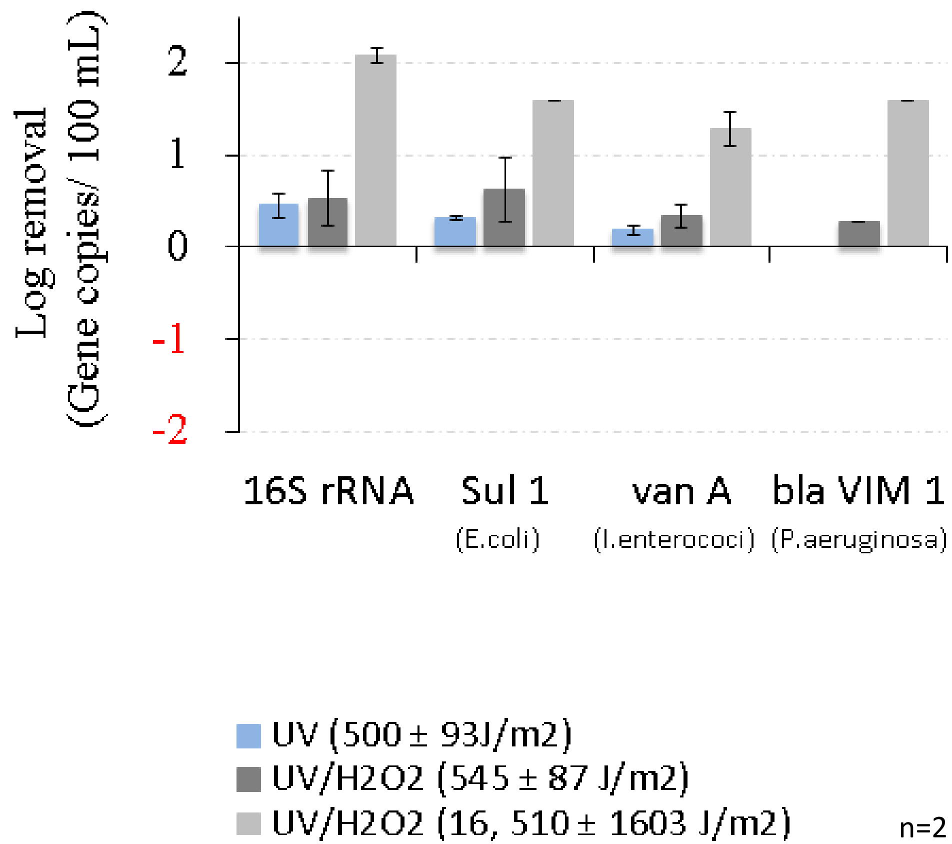
- Limited removal capabilities for imipenem resistance gene (BlaVIM1) might be genome size or bacterial community dependent
- Incomplete reduction in Gene copies -> Hypothesis: Transfer of ARGs by autochthonous bacteria

Performance of UV disinfection and AOP

Abundance of FIB before and after treatment



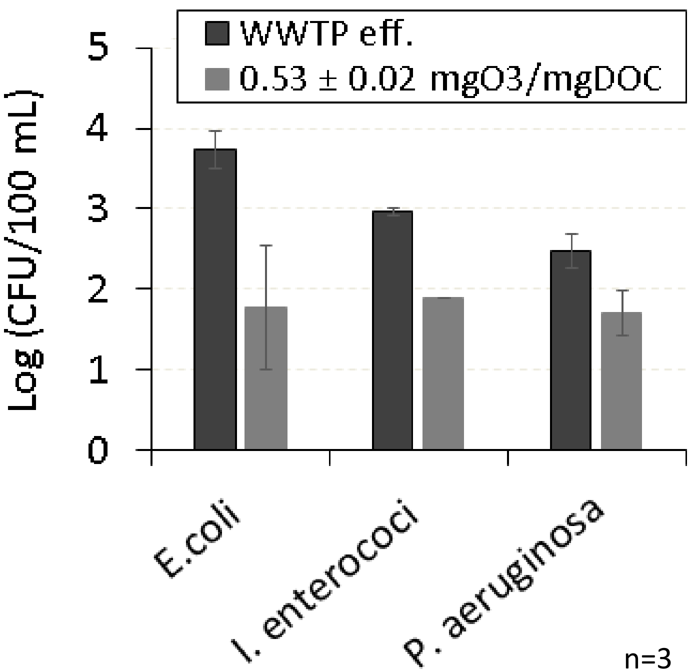
Elimination of target genes



- Incomplete reduction of FIB and negligible elimination of genes at typically applied UV dosages -> Hypothesis: selection effect for non- resistant organisms?
- Significantly greater abatement in number of gene copies at the highest applied UV fluence in AOP

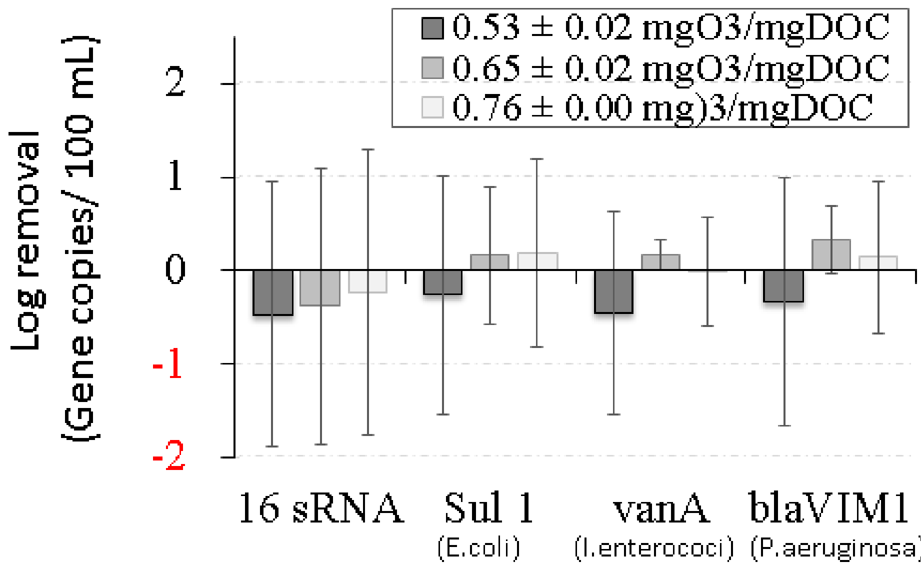
Performance of Ozonation

Abundance of FIB before and after treatment



n=3

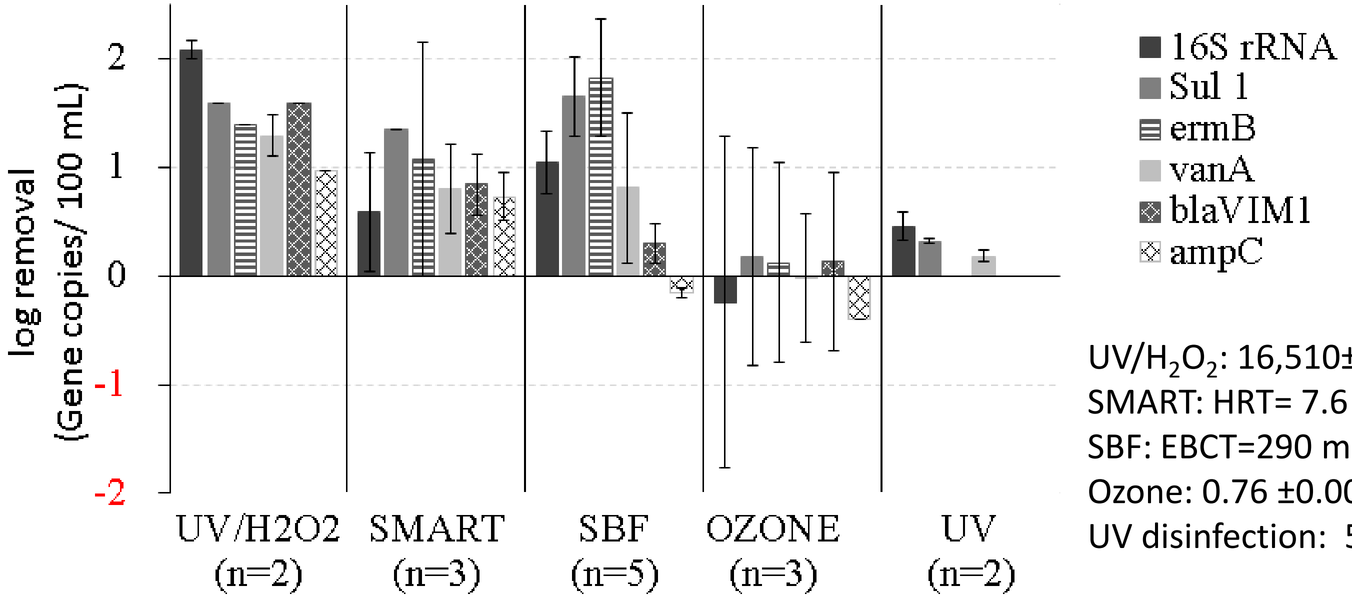
Elimination of target genes



n=3

- Ozone dosages recommended for removal of micro-pollutants are not sufficient for wastewater dis.
- No reduction in gene copies ->Hypothesis: release of free DNA into the environment and its incorporation into bacterial population of the environment

Conclusions



UV/H_2O_2: 16,510±600 J/m^2, 9.5±0.3 mgH_2O_2/L
SMART: HRT= 7.6 d
SBF: EBCT=290 min
Ozone: 0.76 ±0.00 mgO_3/mgDOC
UV disinfection: 500 ±93 J/m^2

- AOP show the greatest efficiency in the reduction of target genes
- Almost identical abatement of Sul1, ermB and vanA genes in SMART and SBF, however BlaVIM1 and ampC show resistance through SBF
  - -> Bacterial species and genome size dependent
  - -> Transfer of ARGs by autochthonous bacteria
- Ozone and UV have no effect on elimination of genes
  - -> Selection effect on non-resitant bacteria
  - -> Release of free genes

Future research

Determinate operational conditions relevant for ARB and ARGs reduction

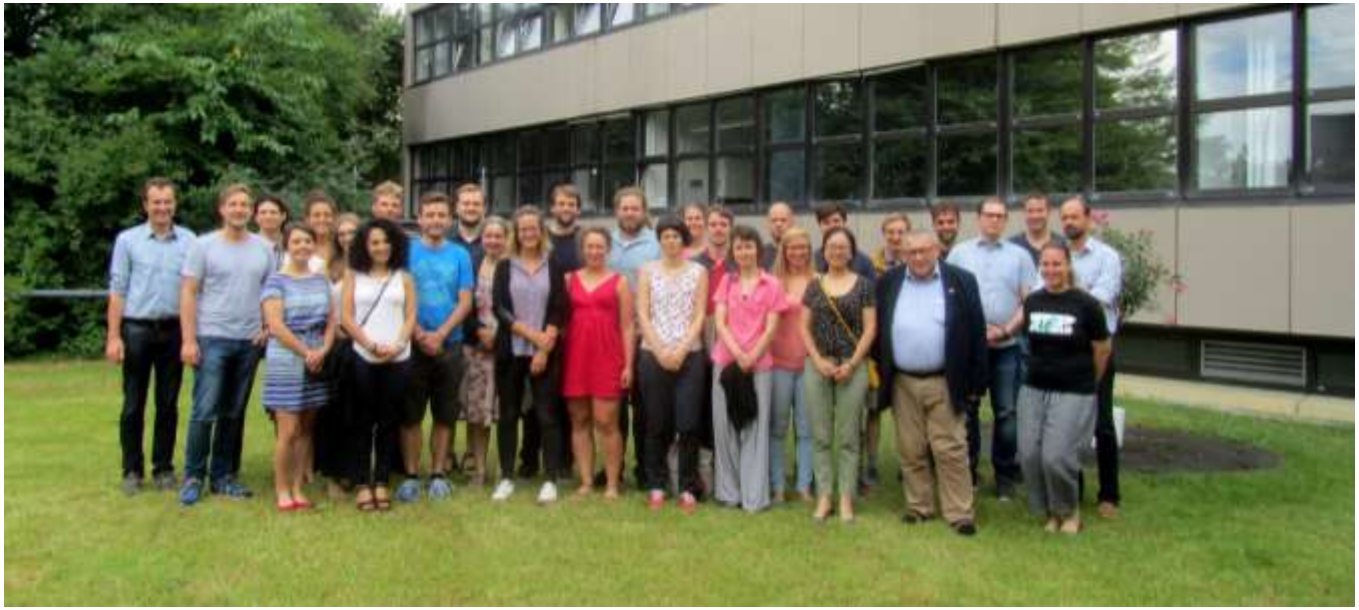
↑

Identify removal mechanism of ARB and ARGs

↑

Optimisation of ARGs detection:
discriminate between live (cell with intact cell membrane) and dead cells and cell activity

Thank you for your attention



Soňa Fajnorova, Uwe Hübner, Bastian Herzog, Johann Müller, Karin Hellauer, David Miklos, Jörg E. Drewes, Jiří Wanner

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

TECHNISCHE UNIVERSITÄT MÜNCHEN

Bayerisches Staatsministerium der Finanzen, für Landesentwicklung und Heimat