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COMBINED ADVANCED PROCESSES IN WASTEWATER TREATMENT FOR REDUCTION OF ANTIBIOTIC RESISTANT BACTERIA

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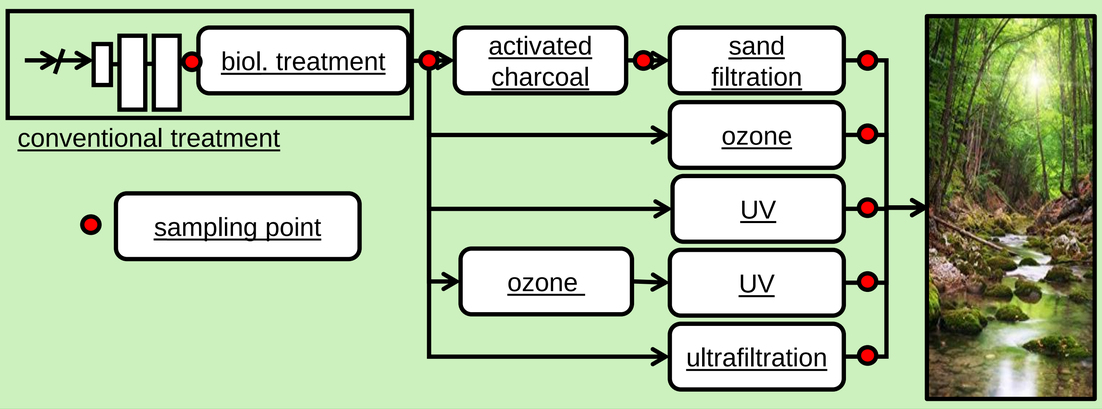
CONVENTIONAL TREATMENT

- Upon international studies , the current conventional wastewater treatment is not suited to eliminate unwanted bacteria as well as antibiotic resistance genes and contributes to their spread to the environment.
- Calculations with real data from the wastewater treatment plant (WWTP; **440.000 P.E. and 80.000-100.000m³/d)**: **each day 2.35x10^{14} facultative-pathogenic bacteria and 9.69x10^{11} copies of antibiotic resistance genes reach the environment,** despite activated charcoal treatment and sand filtration at the WWTP**.**
- **Theoretically does every 23rd bacterium released contains one antibiotic resistance gene!!!**

METHODS

- Normalization to **100 mL** water.
- Quantification of facultative-pathogenic bacteria and clinically relevant resistance genes using qPCR.
- For validation of molecular methods, cultivation based assays were performed with taxonomic-selective agar plates.



Molecular biology - qPCR

- Filtration through 0.2 µm membrane **(600 mL -20 L).**
- Considering **only living bacteria with intact cell walls** (utilizing PMA).

Microbiology - Cultivation

- Filtration through 0.45 µm nitrocellulose membrane **(0.1-750 mL)** depending on sample point and selective agar.
- Serial dilution for each sample.
- Local sample preparation, incubation, and evaluation at the WWTP.

CONCLUSION

- **Ozonation** is capable to reduce microbiological contaminations including ARGs/ARBs.
- **Reduction efficiency** is depending on the ARG carrying bacteria.
- Additional **UV-treatmen**t demonstrated a **synergistic benefit with ozone**.
- Both, increasing of ozone concentration as well as ozone contact time have an **additional benefit in a real WWTP.**
- **Increasing ozone concentration is more effective than prolonged contact time.** Here the production of chemical transformation products is a critical issue.
- **High risk antibiotic resistances** were reduced below the LOD by every parameter variation.
- Reduction efficiencies were as high as analyzed for **ultrafiltration** in some cases.
- **Cultivation based experiments** confirmed the molecular biological methods, but were less distinct.
- **Reduction does not mean elimination: Post-processing regrowth can occur.**

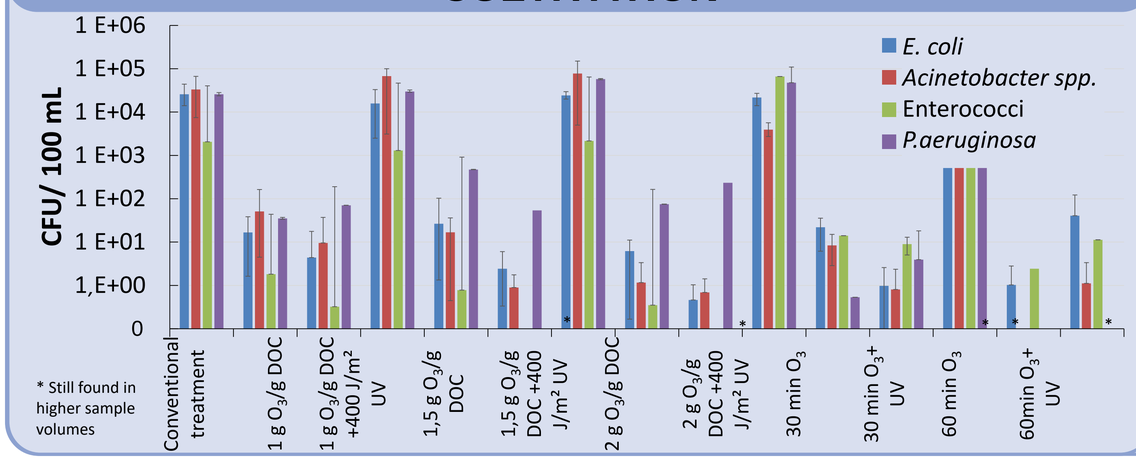
MOTIVATION

- **Ultrafiltration yield the highest reduction** values followed by a combination of ozone and UV- treatment.
- **Ozone and UV- treatment in combination can be considered as an alternative for membrane filtration**, although their efficiency by initial parameters were not as high.
- **Ozone contact times and ozone concentrations/g DOC with UV were increased for further reduction increase of ARB/ARG**.
- **Ozone concentration** was increased to **1, 1.5, and 2.0 g ozone/g DOC; ozone contact time** increased from **5, 30, and 60 minutes;** each with an additional **UV- irradiation of 400 J/m²**.

REDUCTION THROUGH ADVANCED TREATMENT COMBINATION AND VARIATION

		facultative-pathogenic bacteria					Antibiotic resistance genes						
		Enterococci	*A. baumannii*	*E. coli*	*K. pneumoniae*	*P.aeruginosa*	sul1	ermB-1	blaTEM	tetM	ctx-M-32	blaVim2	blaOxa48
conventional treatment	Raw wastewater												
	Biol. treatement Sedimentation	**2,25E+04**	**7,41E+02**	**1,04E+04**	**7,50E+03**	**8,58E+02**	**4,35E+06**	**6,89E+04**	**1,83E+05**	**4,06E+04**	**1,31E+04**	**6,09E+03**	**9,73E+03**
concentration variation	**Ozone (5 min 1 g/g DOC)**											**LOD**	
	Ozone +UV (5 min 1 g/g DOC und 400 J/m²)											**LOD**	**LOD**
	Ozone (5 min 1.5 g/g DOC)	**LOD**	**LOD**		**LOD**	**LOD**						**LOD**	**LOD**
	Ozone +UV (5 min 1.5 g/g DOC /400 J/m²)	**LOD**	**LOD**		**LOD**	**LOD**						**LOD**	**LOD**
	Ozone (5 min 2 g/g DOC)	**LOD**	**LOD**		**LOD**	**LOD**						**LOD**	**LOD**
	Ozone +UV (5 min 2 g/g DOC /400 J/m²)	**LOD**	**LOD**	**LOD**	**LOD**	**LOD**						**LOD**	**LOD**
Ozone contact time prolongation	**Ozone (30min 1 g/g DOC)**											**LOD**	
	Ozone+ UV (30min 1 g/g DOC)/ 400J /m²				**LOD**	**LOD**						**LOD**	**LOD**
	Ozone (60 min 1 g/g DOC)				**LOD**	**LOD**				**LOD**		**LOD**	**LOD**
	Ozone+ UV (60min 1 g/g DOC)/ 400 J/m²			**LOD**	**LOD**					**LOD**		**LOD**	**LOD**
	Ultrafiltration	**LOD**	**LOD**	**LOD**	**LOD**						**LOD**	**LOD**	**LOD**

CULTIVATION



* Still found in higher sample volumes

- **Reduction efficiencies were improved by elevated ozone concentrations and contact times, in most of the cases.**
- Ozone concentration of **1.5g/g DOC resulted in elimination of** most **facultative-pathogenic bacteria.**
- Effect of a subsequent **UV- irradiation** is independent of previous ozone treatment.
- Cultivation experiments support the more distinct qPCR results.

Caption

5,00E+06 — biol. treatment / reference value

1 Log unit increase

2 Log unit reduction

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