

# OFFLINE AND ONLINE FLOW CYTOMETRIC MONITORING OF MICROBIOLOGICAL CHANGES ALONG A TREATMENT TRAIN (MULTI-REUSE)

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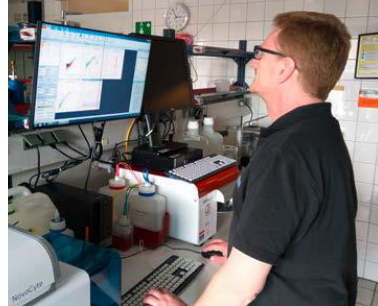
# Project team and acknowledgement

Funding agency: **BMBF (German Federal Ministry of Education and Research)**

IWW team:



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Project partners:



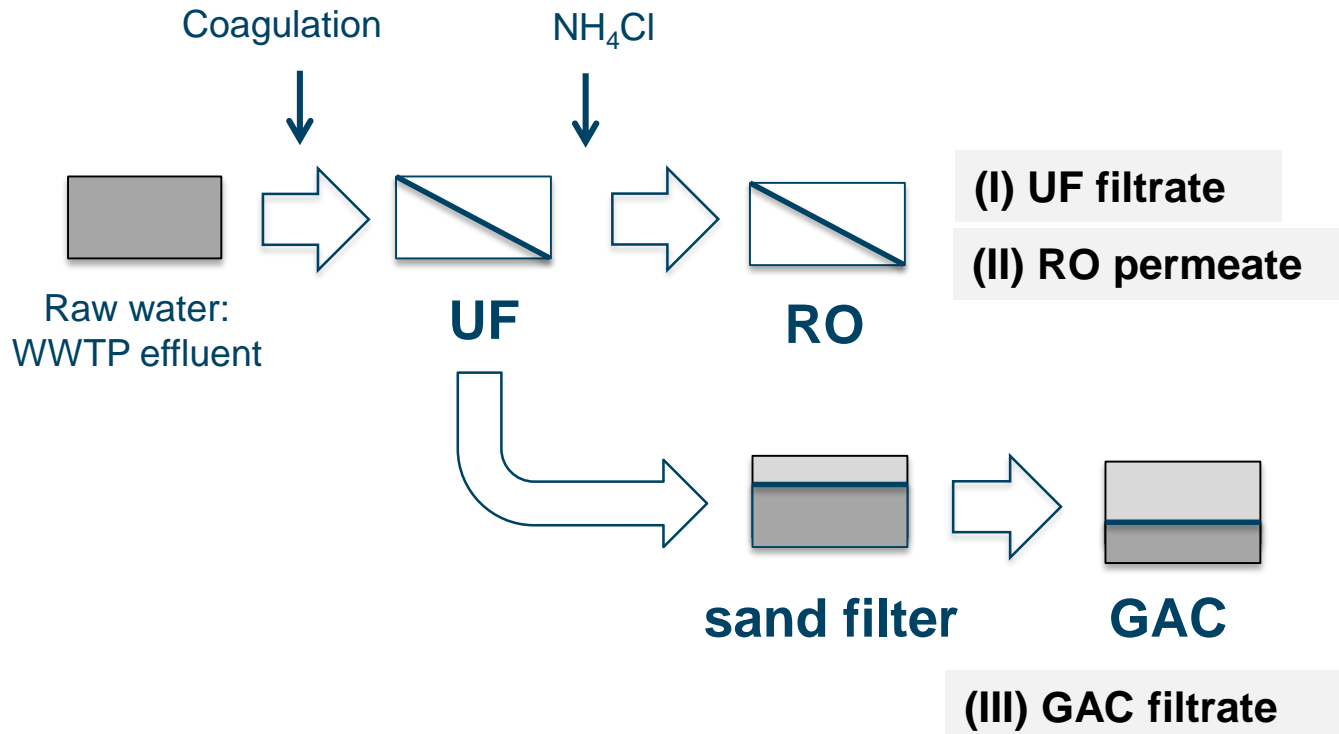
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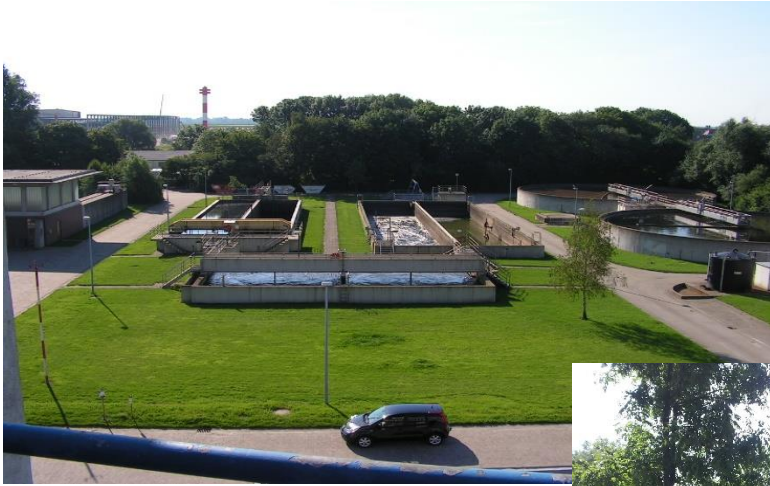
*Offen im Denken*



# Treatment train overview: 3 water qualities



# MULTI ReUse



Source: town of Nordenham



- Test phase (2017/18): variation of operational parameters
- Demonstration (2019): stable operation

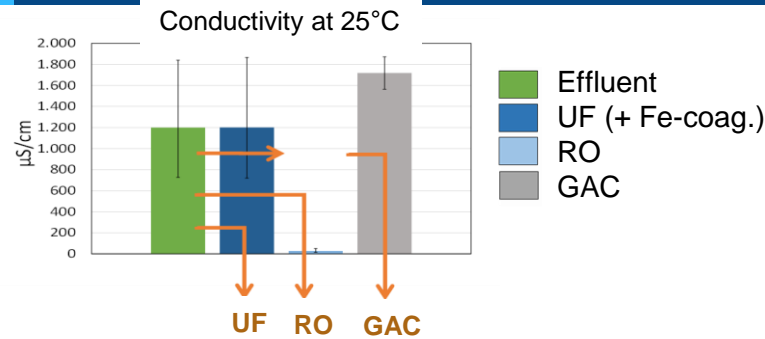




# Water reuse pilot plant at WWTP Nordenham



# Water qualities: MULTI-ReUse

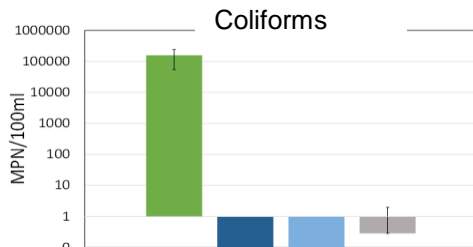
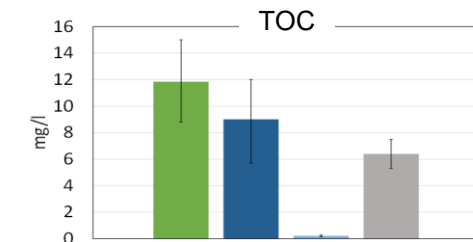


## ReUse water 1 (after UF)

- particle-free, hygienically impeccable
- e.g. for washing and cooling processes

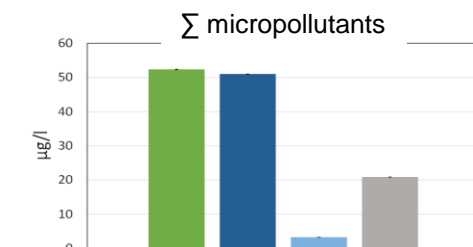
## ReUse water 2 (after RO)

- Very low in ions, organic micropollutants and TOC
- e.g. for cooling processes or special use

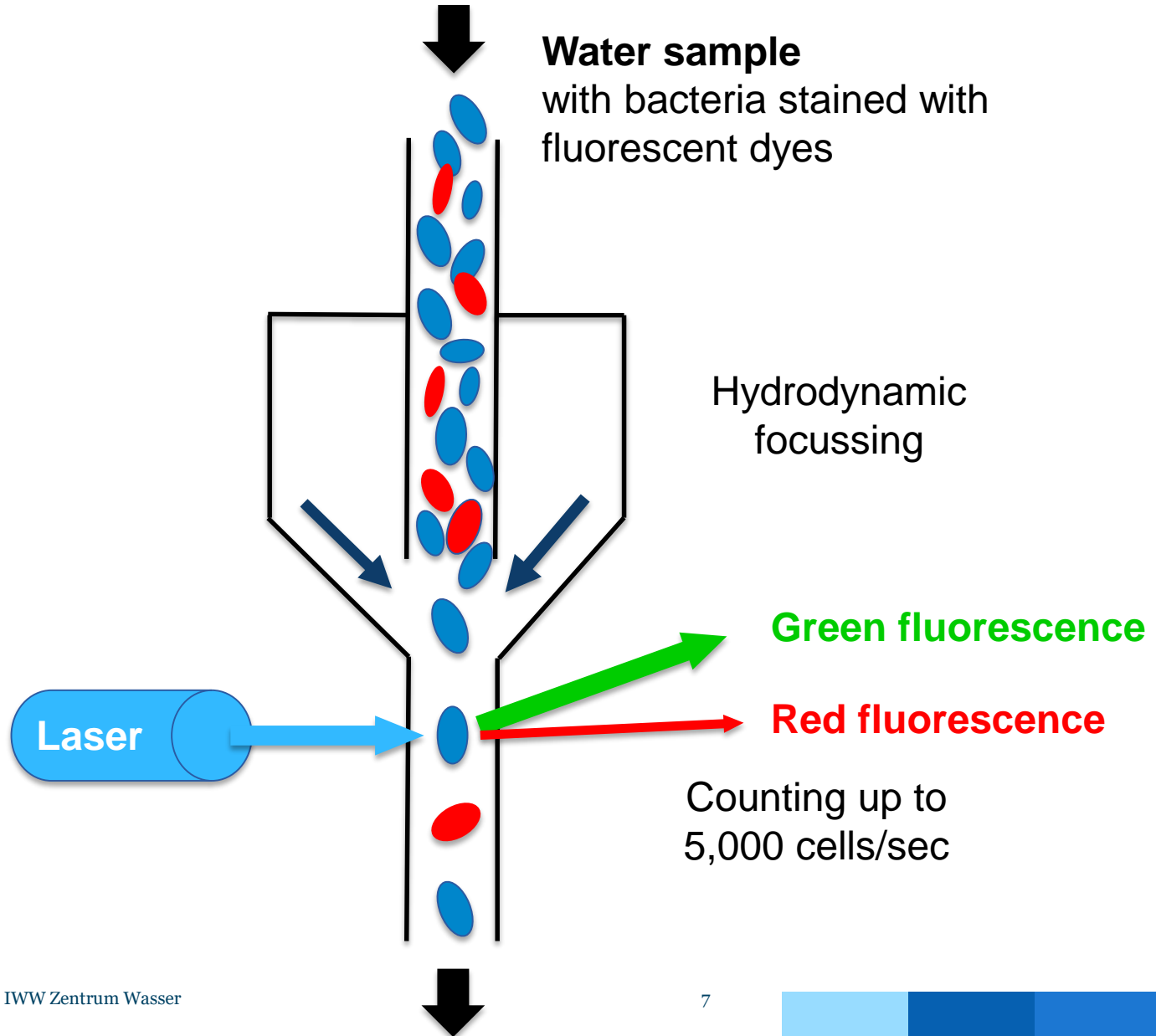


## ReUse water 3 (after GAC)

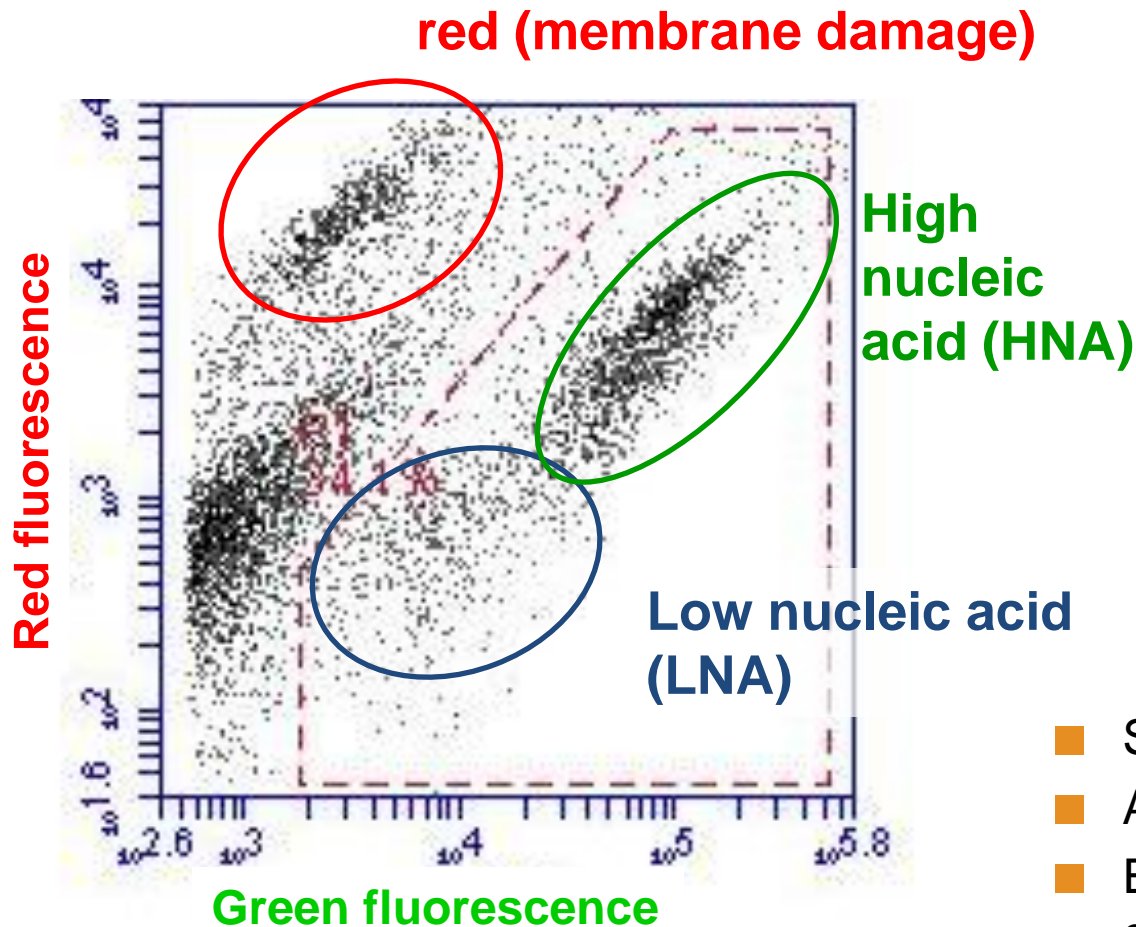
- particle-free, hygienically impeccable and stable
- low in micropollutants
- e.g. for irrigation or infiltration



# Principle of flow cytometry



# Example of flow cytometric dot blot



- Staining with fluorescent dyes
- Analysis time: 15 min
- Every signal in gated area = one bacterium
- LNA: bacteria with little DNA
- HNA: bacteria with more DNA

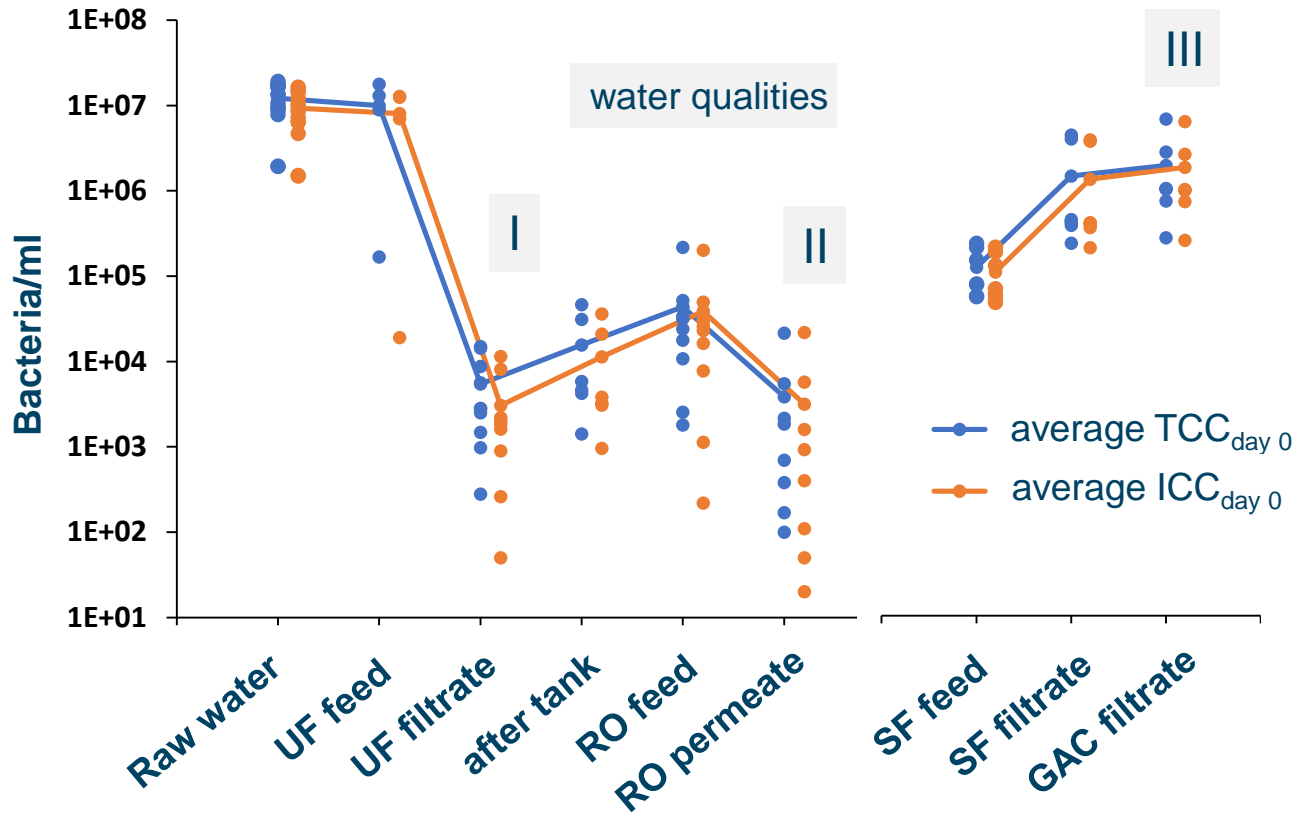


# Hygienic status after treatment

		Raw water		UF filtrate	
		Average	Max./Min.	Average	Max./Min.
<i>E. coli</i>	MPN/100 ml	79,100	198,600/20,600	0	0/0
Enterococci	MPN/100 ml	164,900	2,200,000/600	0	1/0
<i>Clostridium perfringens</i>	MPN/100 ml	64,300	810,000/4.200	0	0/0
<i>Legionella</i> spp.	MPN/100 ml	> 100	> 100/0	0	0/0
Colony counts 22°C	CFU/ml	24,600	96,000/5,800	2	10/0
Colony counts 36°C	CFU/ml	42,600	290,000/6,900	120	700/0

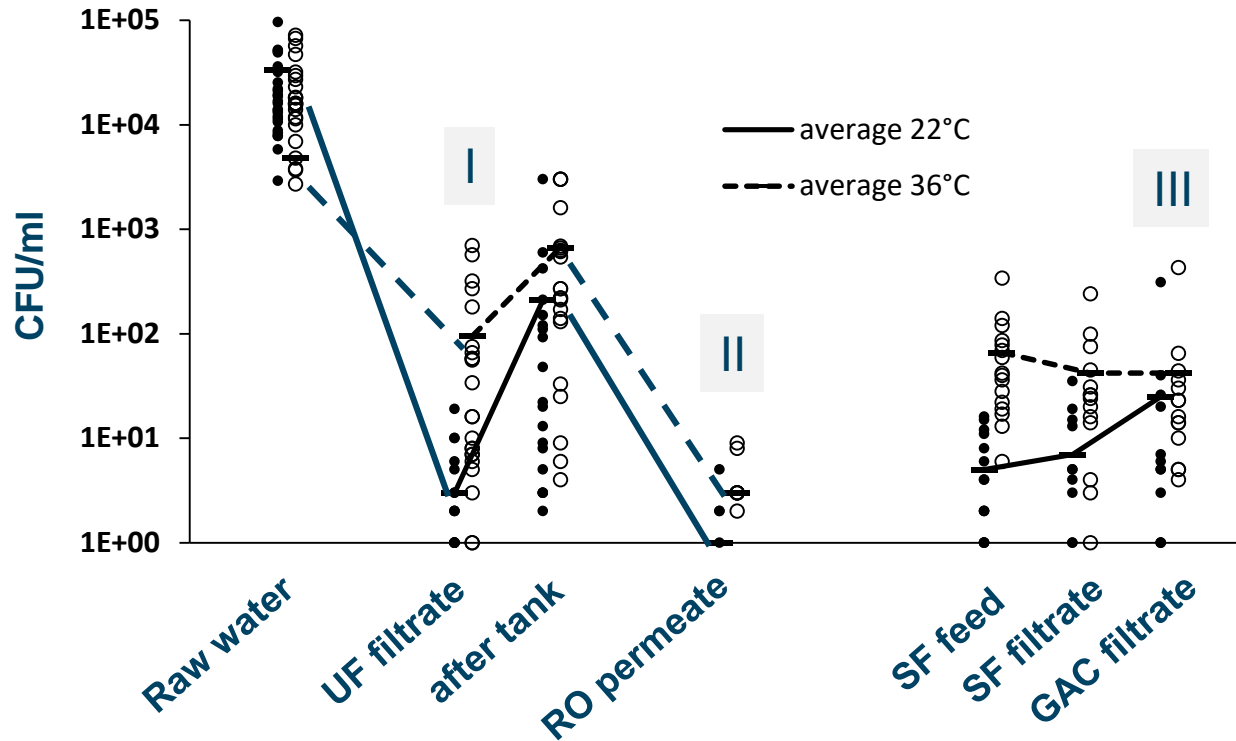
➤ Already the first barrier assures a good hygienic status.

# Changes in bacterial numbers over treatment



➤ Concentrations of bacterial numbers varied greatly over treatment train.

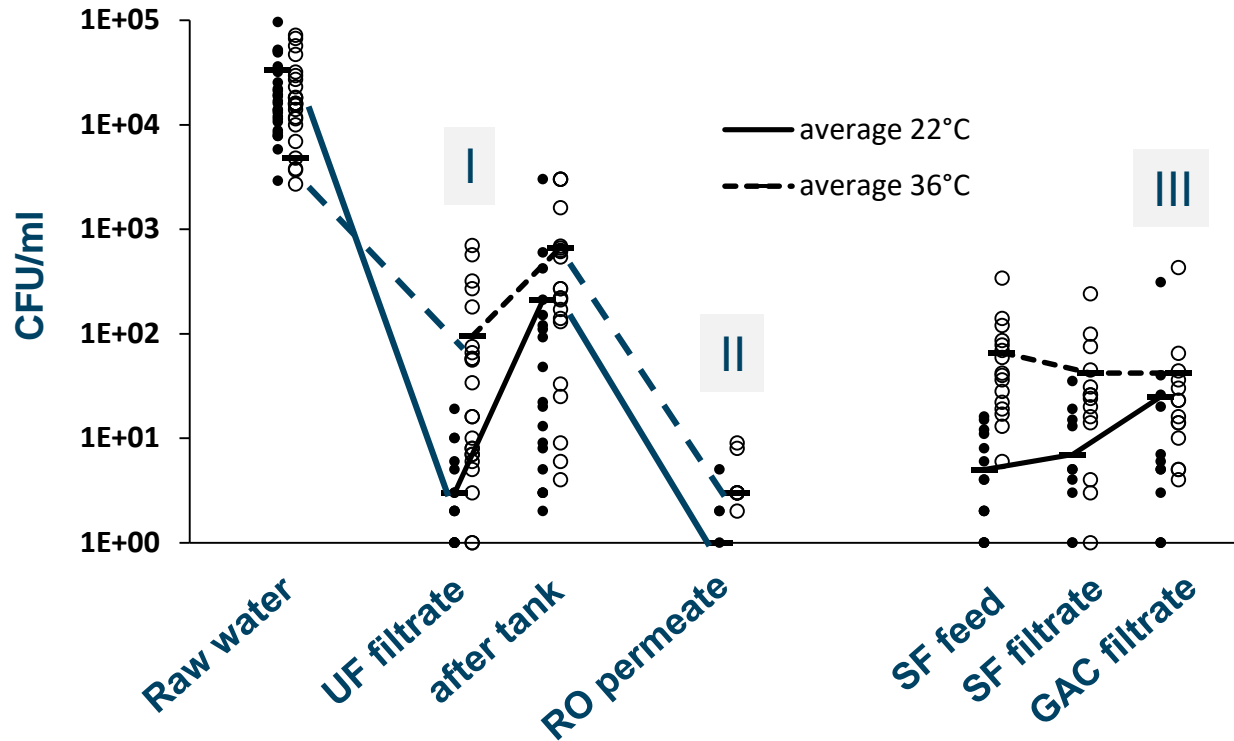
# Changes in bacterial numbers over treatment



% culturable	Raw water	UF filtrate	after tank	RO permeate
22°C	0.361	0.098	1.853	0.031
36°C	0.351	3.103	5.920	0.094

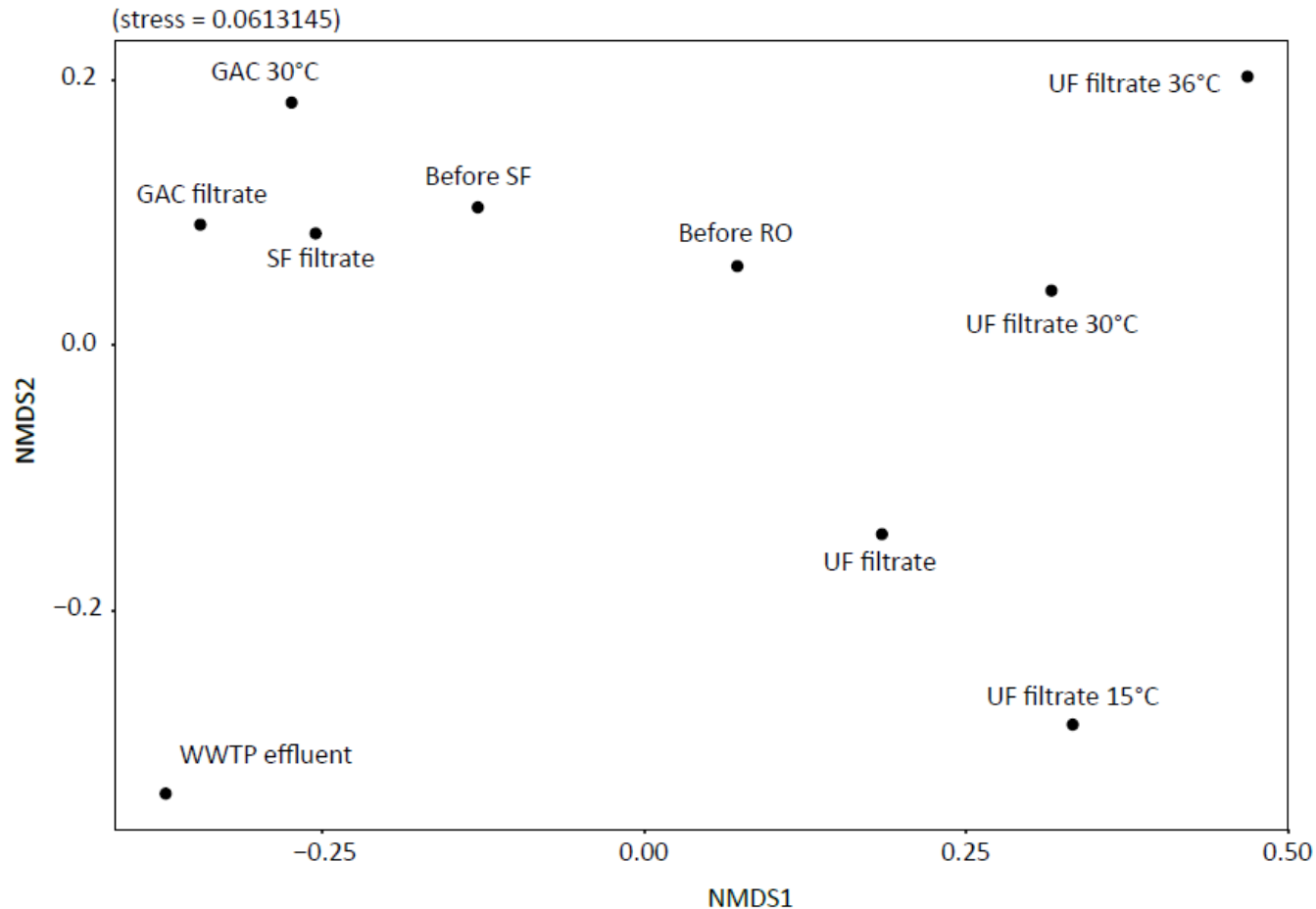
SF feed	SF filtrate	GAC filtrate
0.005	0.001	0.002
0.069	0.004	0.003

# Changes in bacterial numbers over treatment



- Trend in colony counts follows the trend of ICC
- Distortion however as % culturable bacteria fluctuate over treatment train

# Dissimilarity (Bray-Curtis)



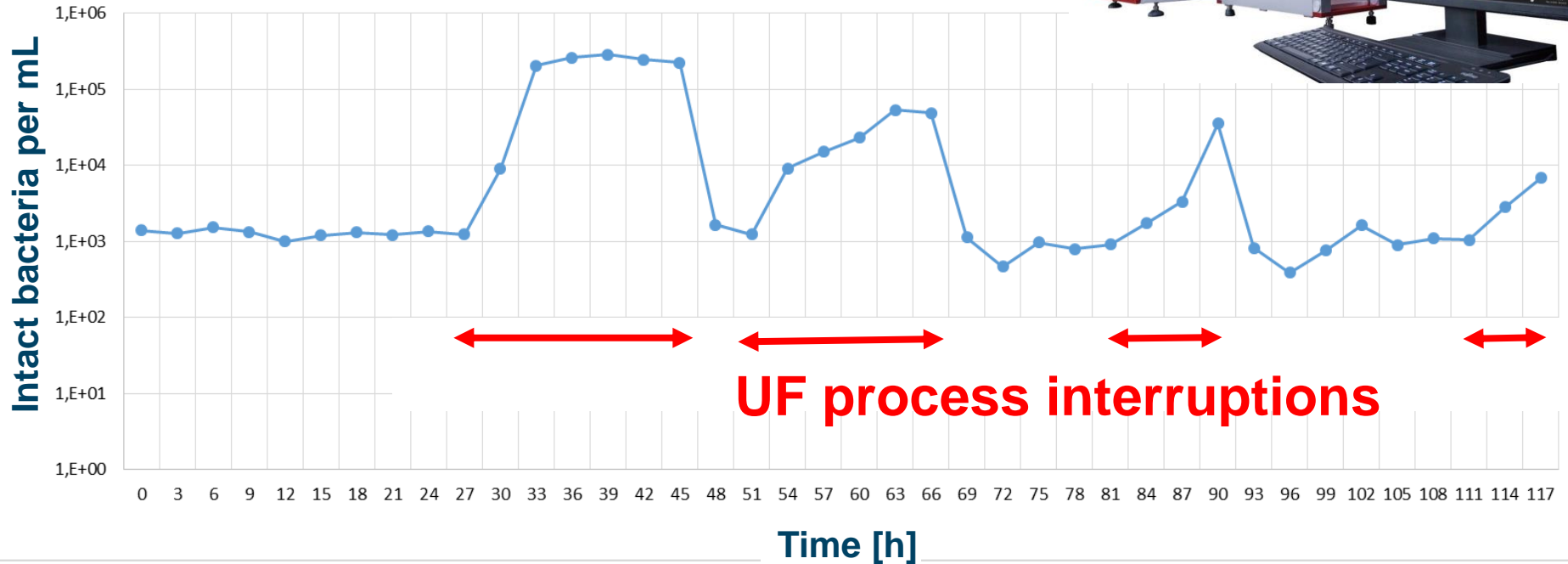
- Treated water is clearly distinct from WWTP effluent.
- Temperature is an important determinant for the composition of a regrowing population.



# Automated monitoring of intact bacteria

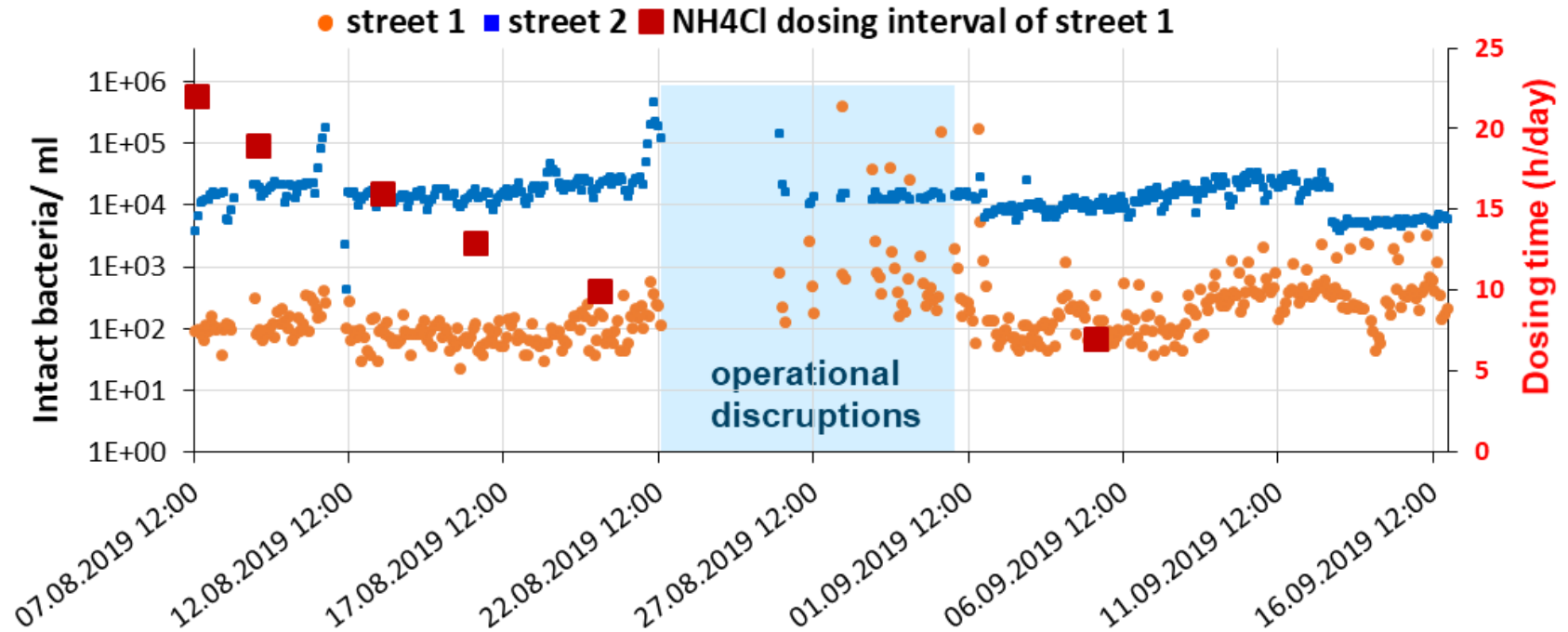


# Online flow cytometry



- UF interruptions lead to increased bacterial numbers within approx. 1.5 h.
- Online FCM is suitable as an early warning tool for detecting process failures.

# Online flow cytometry



- High data resolution of online FCM allowed process optimization.
- Monochloramine dosing time of 13 h was chosen to suppress regrowth.

# Regrowth potential



**Day 0**

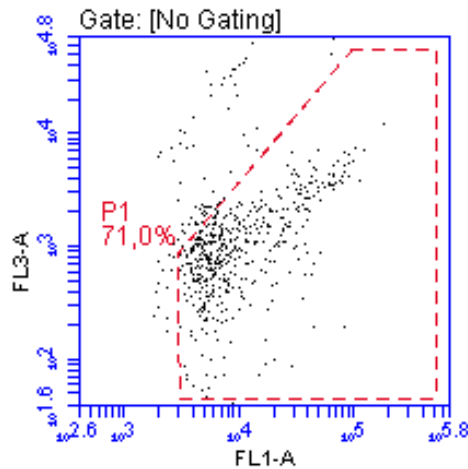
**22°C**



Incubation in AOC-free vessels over 7 days.  
Sample volume: 20 mL



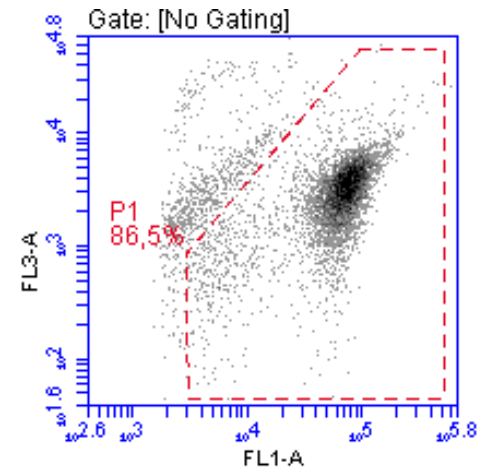
**Day 7**



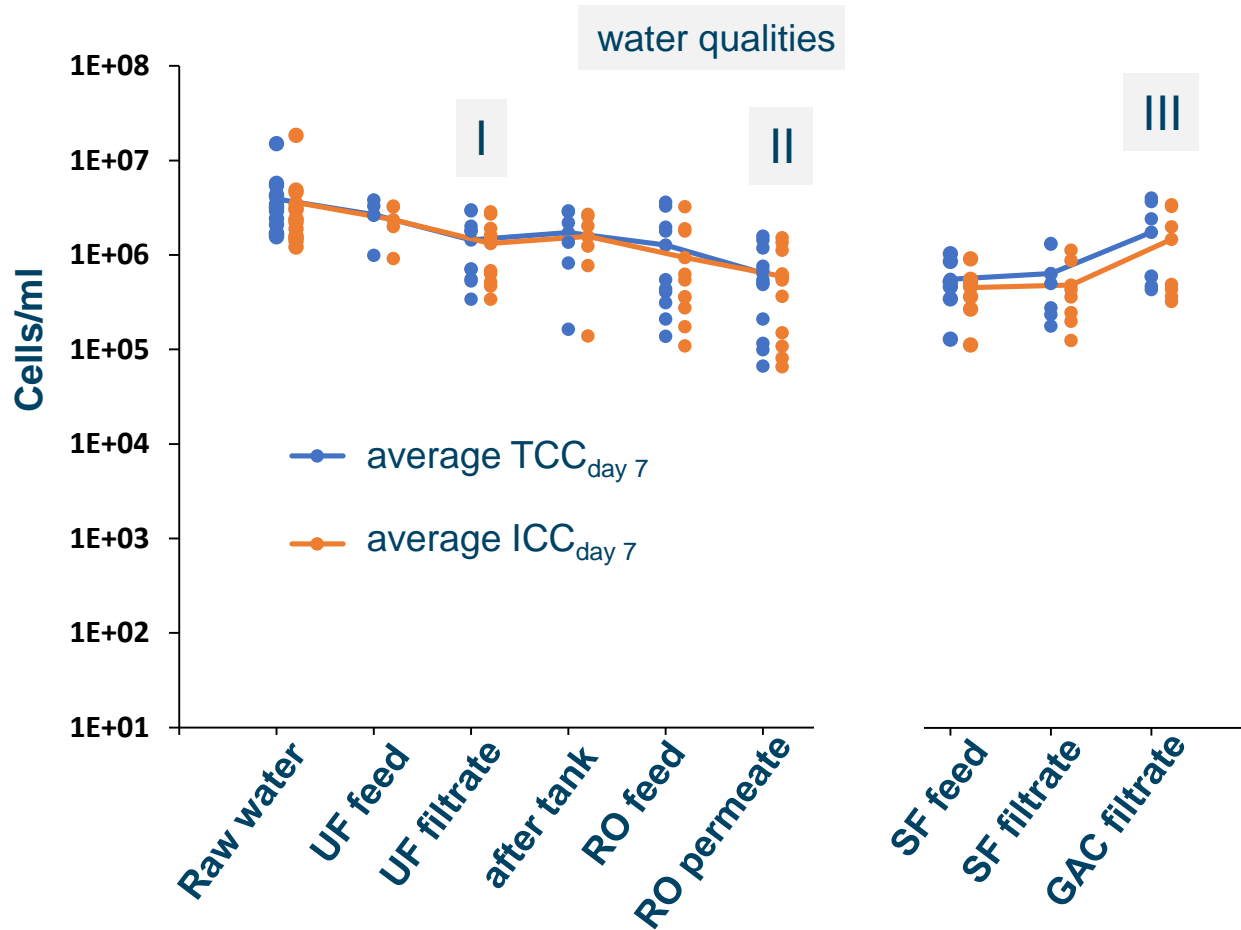
**Regrowth potential**

**ICC (Day 7)**

**ICC (Day 0)**

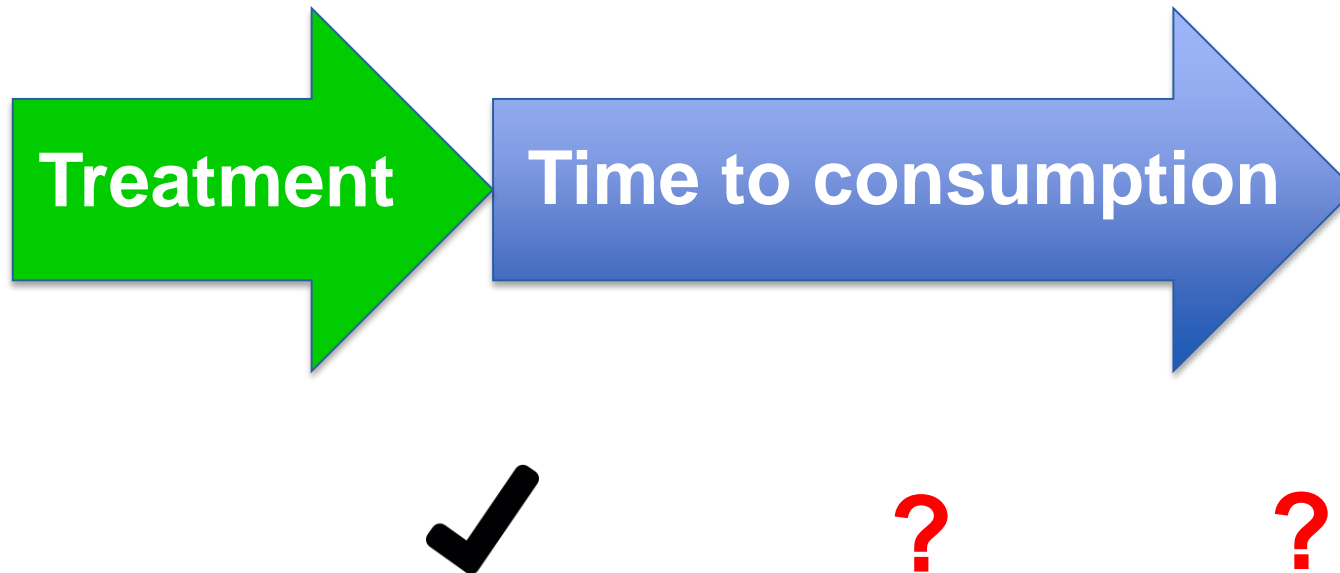


# Change of regrowth potential



- Day 7 values only decrease moderately.
- Reference: stagnated drinking water in a plumbing system can easily contain 10<sup>6</sup> bacteria/ml.





- Microbiological water quality is a continuum and is highly dynamic.
- Depending on the application, the regrowth potential of relevant pathogens should be addressed (e.g. *Legionella* in water destined for a cooling tower)