Karlsruhe Institute of Technology

Institute of Functional Interfaces (IFG)

Microbiology of Natural and Technical Surfaces Department

Does copper material induce VBNC state in drinking water biofilm bacteria?

Jungfer, C., **Varela Villarreal, J., Brändle, K., Friedrich, F., Obst, U., Schwartz, T.**

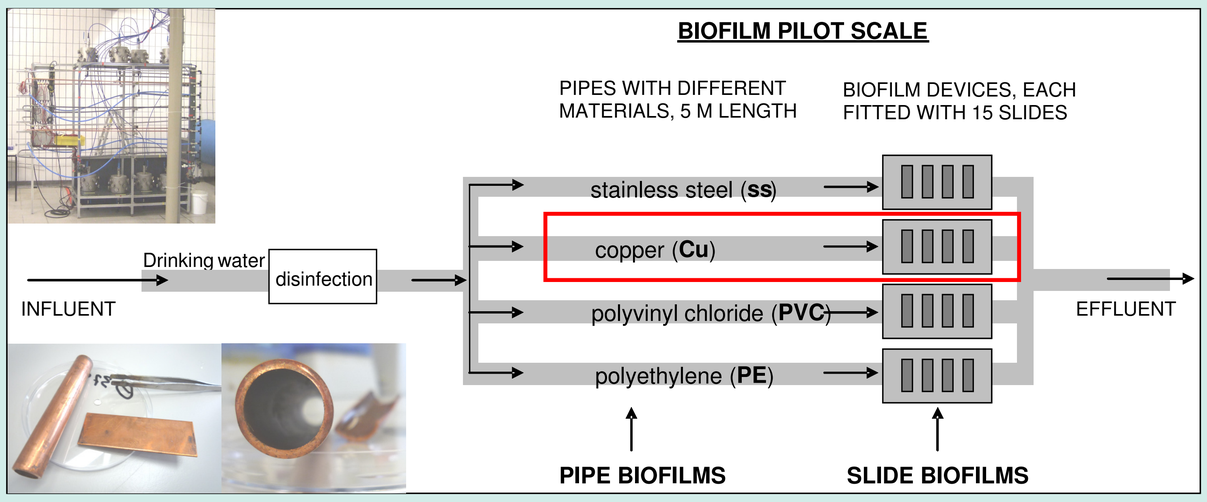
E-mail: christina.jungfer@kit.edu

BACKGROUND AND EXPERIMENTAL APPROACH

Biofilms from drinking water pipelines are a possible niche for all kinds of bacteria, including pathogens, and may be responsible for contamination of drinking water systems. In general, a clear understanding of survival, regeneration and regrowth of bacteria in biofilms is of considerable value.

To get a deeper look in this problem, drinking water biofilms from different waterworks (K, M, F, S) were investigated in a modular pilot scale using different pipe materials including copper. Three months old biofilms were investigated with molecular biological and microscopy methods.

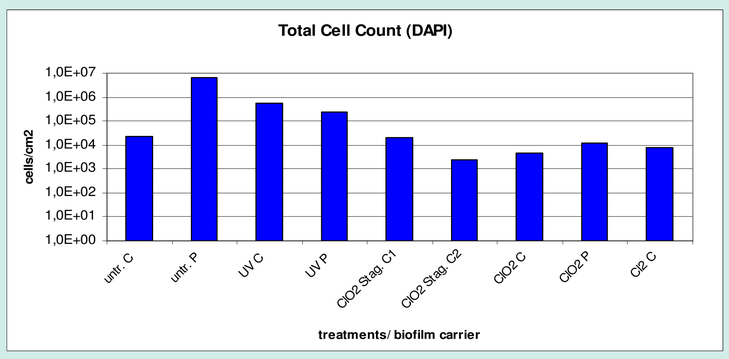
When copper was used as pipe material, remarkable results and differences to other materials were found in biofilms.

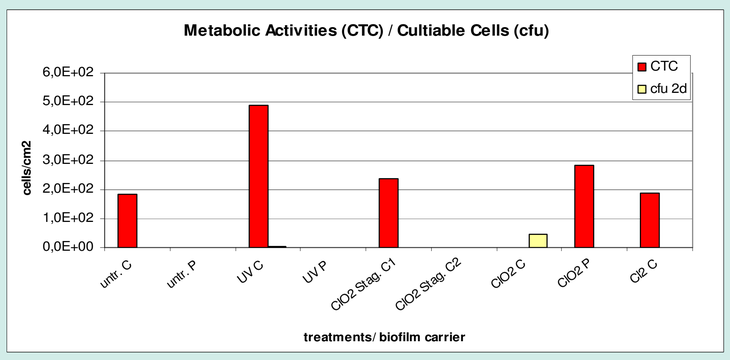


RESULTS

High **TOTAL CELL COUNTS** (10^4 up to 10^6 cells/cm^2) were detected without disinfection and after UV disinfection. After chemical disinfection treatment 10^3 up to 10^4 cells/cm^2 were observed.

But low **METABOLIC ACTIVITIES** and no or low numbers of **CULTIVABLE CELLS** (cfu) were found in these biofilms indicating a high number of VBNC. Similar results were detected with other materials (data not shown).





Treatments: untr.: untreated; UV: UV disinfection; ClO_2: chlorine dioxide disinfection; Cl_2: chlorine disinfection; Stag.: stagnation. **Biofilm carrier:** c: coupons; p: pipes

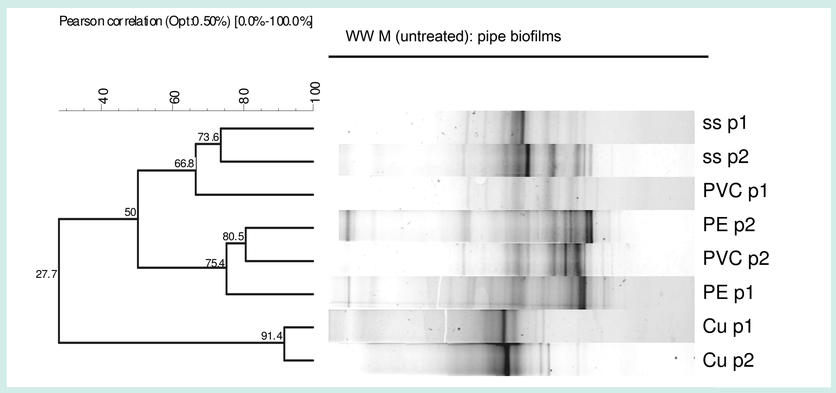
When stress and vitality markers on RNA level were investigated with **GENE EXPRESSION ANALYSIS**, a distinct *recA* induction, which is responsible for the dark repair, was found in biofilms from copper material after UV disinfection.

- **UV disinfection/oxidation:** up-regulation of *recA*-mediated dark repair in natural biofilms.
- **ClO_2 and Cl_2 disinfection:** no *recA* expression.
- **copper:** highest *recA* induction in biofilms.

treatment	WW	biofilm carrier	stainless steel	copper
untreated	K	c	↔	↔
	M	c	↑	n.d.
	M	c	n.d.	↔
	M	p	n.d.	n.d.
UV disinfection	K	c	↑	↔
	F	c	↑	↑
	F	p	n.d.	↑
UV disinfection + 0.3 NTU	K	c	↔	↑↑
UV oxidation	K	c	n.d.	↑↑
chlorine dioxide disinfection	S	c	n.d.	↔
	S	p	↔	↔
chlorine disinfection	K	c	n.d.	n.d.
	S	c	n.d.	↔
	S	p	n.d.	↔

WW = waterworks; c = coupons; p = pipes; ↑ = *recA* induction; ↔ = *recA* ground expression; n.d.: *recA* not detected; NTU= nephelometric turbidity unit.

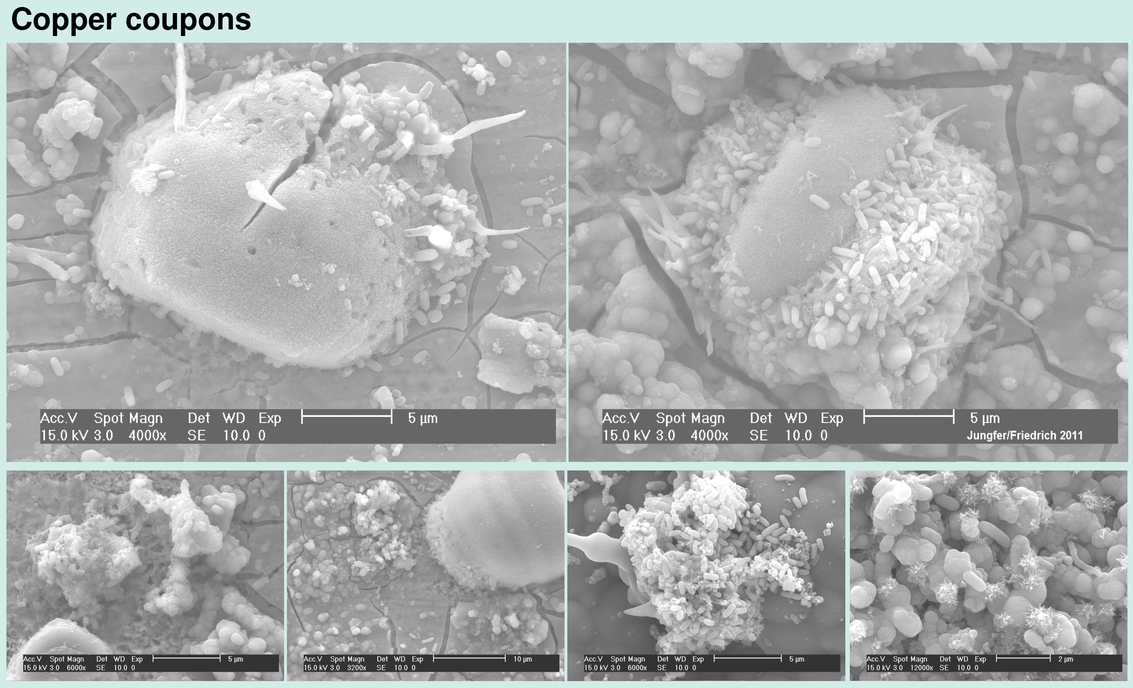
Population analysis by **PCR-DGGE** showed a significant shift in bacterial populations when copper material was used after the different disinfection treatments. In general a decrease in band number was observed.



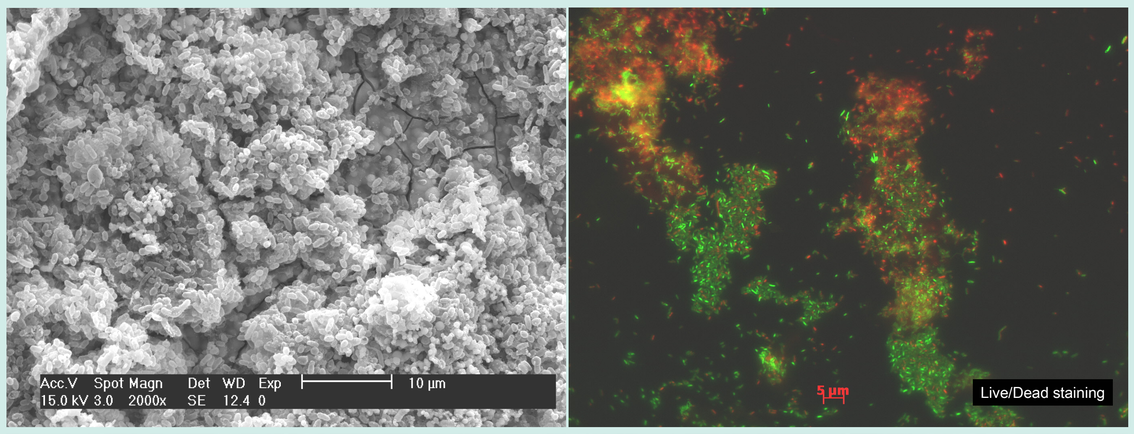
PCR: Polymerase Chain Reaction; DGGE: Denaturating Gradient Gel Electrophoresis.

The use of **ENVIRONMENTAL SCANNING ELECTRON MICROSCOPE** (ESEM) gave us a clear picture of intact cells and mushroom-like biofilm structures on the copper surface. **LIVE/DEAD STAINING** verified clearly the presence of cells with intact cell membranes.

Copper coupons



Copper pipes



At stainless steel surfaces no clear biofilm pictures were observed with the ESEM microscopy (data not shown).

CONCLUSIONS

The presence of natural bacterial communities on copper pipelines were verified under real drinking water conditions after applying modern detection methods.

These results clearly showed that copper surface did not prevent biofilm formation defying the bacteriostatic copper properties theory. After three months in drinking water an intact biofilm was found on copper material with populations diverse to other materials. Though almost no cultivable bacteria were found, a clear gene expression activity was detected

All these results indicate the presence of VBNC bacteria on copper.

KIT – University of the State of Baden-Wuerttemberg and National Research Center of the Helmholtz Association

This work was funded by a grant of the BMBF.

www.kit.edu