



# Verification of the efficiency of chemical disinfection and sanitation measures in in-building distribution systems

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## ABSTRACT

Previous investigations of biofilms, generated in a silicone tube model have shown that the number of colony forming units (CFU) can reach  $10^7/\text{cm}^2$ , the total cell count (TCC) of microorganisms can be up to  $10^8$  cells/ $\text{cm}^2$ .

The present study focuses on the situation in in-building distribution systems. Different chemical disinfectants were tested for their efficacy on drinking water biofilms in silicone tubes: free chlorine (electrochemically activated), chlorine dioxide, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), silver, and fruit acids.

With regard to the widely differing manufacturers' instructions for the usage of their disinfectants three different variations of the silicone tube model were developed to simulate practical use conditions..

First the continuous treatment, second the intermittent treatment, third the efficacy of external disinfection treatment and the monitoring for possible biofilm formation with the Hygiene-Monitor. The working experience showed that it is important to know how to handle the individual disinfectants. Every active ingredient has its own optimal application concerning its concentration, exposure time, physical parameters like pH, temperature or redox potential. When used correctly all products tested were able to reduce the CFU to a value below the detection limit. Most of the active ingredients could not significantly reduce the TCC/ $\text{cm}^2$ , which means that viable microorganisms may still be present in the system. Thus the question arises what happened with these cells? In some cases SEM pictures of the biofilm matrix after a successful disinfection still showed biofilm residues. According to these results, no general correlation between CFU/ $\text{cm}^2$ , TCC/ $\text{cm}^2$  and the visualised biofilm matrix on the silicone tube surface (SEM) could be demonstrated after a treatment with disinfectants.

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## Introduction

Biofilms are very successful biocoenosis, in which microbial life is embedded and protected in a matrix consisting of extracellular polymeric substances (EPS). Nearly all microorganisms live in such synergetic communities. This living form facilitates a cohabitation of different species in various biotopes (Flemming and Wingender, 2001, 2002). Biofilms can be generated in all interfaces between different phases. Growth and formation is a specific process and is divided into three phases: attachment, colonization and growth. When the biofilm has reached a plateau phase a biological and chemical balance of regeneration and degradation is achieved. With local hydrolysis in the EPS or the water flow microorganism or even components of the biofilm matrix can be released into the water phase (Costerton, 1995; Costerton et al., 1999).

In water systems the possible contamination with facultative pathogens like *Pseudomonas aeruginosa* or *Legionella pneumophila* poses be a higher risk of infection especially for immunocompromised people like elderly persons or children (Anaissie et al., 2002; Exner et al., 2005; Hall-Stoodley and Stoodley, 2005; Langsrud et al., 2003; Rahal and Urban, 2000; Reuter et al., 2002; Trautmann et al., 2001).

The problems with biofilms in drinking water distribution systems and attached systems are becoming more and more relevant (Donlan and Costerton, 2002; Exner et al., 2005). In *in-building distribution systems* they can lead to contaminations of the drinking water. Microorganisms organised in biofilms can enter the drinking water installations during water abstraction and purification or working and repairing of plumbing fixtures. Another problem is the possibility of retrograde contamination at the tapping point or carelessness of people, in general (Block, 1992; LeChevallier et al., 1987; Nagy and Olsen, 1985).

Exner et al. (2007) describe that *in-building distribution systems* in medical facilities are often not regarded as a potential origin of nosocomial infections. Critical points in an indoor installation are e.g. longer supply lines, stagnation sectors, depositions, dead

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ends or warm water reservoirs. Since the enactment of the German Drinking Water Ordinance in 2001, hospitals and other public facilities have the responsibility for high quality of drinking water from their indoor system. This law is compulsory for all operators of water distribution systems (Exner and Kistemann, 2004).

A problem is the detection of a biofilm problem in a drinking water installation. It is only possible to take samples of the pipe system and to analyse it in the laboratory. But it is impossible to see developments in the system. With the in the Institute for Hygiene and Public Health developed “Hygiene-Monitor” investigations are possible without manipulating the drinking water installation. It is a small box with an integrated silicone tube to represents a worse case model in which biofilms can exist. Therefore it is an ideal model to illustrate the success of disinfection measurements if biofilms can be removed or the growth can be inhibited. The Hygiene-Monitor is attached to the end of the system so that the silicone tube is flown through all the time. For analysing pieces of the silicone tube are cut out and evaluated in the laboratory. On the rest of the silicone tube developing is like before.

## Materials and methods

### *Silicone tube model*

The silicone tube model is developed in the Institute for Hygiene and Public Health of Bonn University to generate natural drinking water biofilms (Gebel et al., 2009). The model simulates a drinking water distribution system in which drinking water from the “Wahnachtalsperrenverband” (a barrage) is flowing continuously through silicone tubes with a diameter of 4 mm and a wall thickness of 1 mm. To avoid growth of algae the system is shaded. In less than 50 days a biofilm with more than  $10^6$  CFU/cm<sup>2</sup> is formed by natural water bacteria.

### *Determination of heterotrophic plate count*

From the contaminated silicone tube pieces of 5 cm were cut at regular intervals (once a week), the biofilm matrix was scraped-out, homogenised, aliquoted on R2A-Agar and incubated for 7 days at 20 °C. The number of colony forming units (CFU) was determined by heterotrophic plate count (HPC) and converted in CFU/cm<sup>2</sup>.

### *Determination of total cells*

Determination of the total cell count (TCC) was done with Dapi staining (Bredholt et al., 1999). From the contaminated silicone tube pieces of 5 cm were cut, the biofilm matrix was scraped out, homogenised, Dapi solution was added (10 µg/ml), incubated for 20 minutes in darkness, filtered on a black polycarbonate filter (0.2 µm) and counted under blue fluorescence (Zeiss Axioplan, AxioCam MRc digital camera system).

### *Preparation of scanning electron microscopic pictures*

With a scanning electron microscope (SEM, Leitz-AMR1600; Com.Leitz) pictures with a 1000× magnification of the silicone tube surface before and after disinfection treatment were taken. For preparation the silicone tube were cut in pieces of  $0.5 \times 0.5$  cm, incubated for 2 h in a 25% glutaraldehyde solution, fixed in a 0.2% osmiumtetroxide solution and washed for 30 minutes in sodium-cacodylate buffer. In the last step the preparation was dehydrated in an ascending alcohol series. After drying over night the preparation was sputtered with flour gold (Sputter SCD 005 BAL-TEC AG, Balzers, Liechtenstein).

## *Models for testing chemical disinfectants*

1. Continuous treatment of biofilms over a long period of time (one to three months): the disinfectant is added to a fresh water reservoir with a dosing system (Co. Prominent, Heidelberg, Germany) at a defined concentration according to the German Drinking Water Ordinance from 2001.

In the present investigation, a silicone tube with a one-year-old biofilm with more than  $10^6$  CFU/cm<sup>2</sup> was used in order to reduce the number of CFU. At the same time sterile silicone tubes were used under equal conditions to examine the possible biofilm formation.

2. Intermittent treatment: In a closed circulation system, the disinfectant was added to a biofilm-contaminated silicone tube at the concentration recommended by the manufacturer for a defined period of time and at a constant flow rate (400 ml/min).
3. Additionally, the efficacy of the external disinfection treatment and the monitoring of the potential for biofilm formation were supervised with the Hygiene-Monitor®.

The Hygiene-Monitor is a system to verify the efficacy of external disinfection measures. It is attached to the end of the water distribution system and the integrated silicone tube is flushed with the same treated water like the distribution system.

## Results

### *Long-time treatment with continuous dosing of disinfectants*

The continuous treatment with 0.3 ppm free chlorine (limit value German Drinking Water Ordinance) leads to a reduction of the CFU/cm<sup>2</sup> down to the detection limit after 70 days (Fig. 1). Weekly analysis showed that there is a successive reduction. Even after elimination of the CFU the total cell count was still on a level of  $>10^4$ /cm<sup>2</sup>. SEM pictures showed that there were still biofilm residues on the surfaces, which are potential attachment points for new microorganisms (Fig. 3C). Two times a week the concentration of free chlorine was controlled by DPD, the values had a range between 0.22 and 0.30 ppm.

A treatment with chlorine dioxide at a concentration of 0.2 ppm (limit value German Drinking Water Ordinance) leads to a reduction of the CFU/cm<sup>2</sup> after 70 days, too (Fig. 1). The progression was nearly the same as with free chlorine.

SEM pictures also showed biofilm residues and determination of the TCC demonstrated that there are still more than  $10^3$  cells on a cm<sup>2</sup> after a treatment for 84 days. Two times a week the concentration of chlorine dioxide was controlled by DPD, the values had a range between 0.17 and 0.20 ppm. The investigations were reproduced under equal conditions.

In parallel a sterile silicone tube was treated with the disinfectant. In both cases, with free chlorine and chlorine dioxide, a biofilm formation and a growth of CFU or total cells respectively could be prevented. SEM pictures also showed that a settlement on the tube surface could be avoided.

### *Short-time treatment with a shock dosing*

It is a dose-response relationship of the efficacy of hydrogen peroxide in combination with other active ingredients. H<sub>2</sub>O<sub>2</sub> in combination with silver at a concentration of 10,000 ppm reduces the CFU/cm<sup>2</sup> to the detection limit within 4 h. An increase of the concentration from 1000 to 10,000 ppm showed a higher decrease of the CFU. 1000 ppm H<sub>2</sub>O<sub>2</sub> reduced the CFU by 3 log units within 4 h, 5000 ppm reduced it by 4 log units. The TCC could only be

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