Effect of Retrograde Microbial Contamination on Mobile Drinking Water Systems

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ABSTRACT

The facultative pathogenic bacteria *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* where tested for their ability to contaminate retrogradely mobile drinking water systems (MDWS). Water and biofilm samples were taken regularly from characteristic points in close-to-practice test plants and were analysed for the facultative pathogens according to international standards. Additionally, fluorescence in situ hybridization was applied for detection of *Pseudomonas aeruginosa*. Each of the facultative pathogens carries the potential to contaminate retrogradely MDWS. Retrograde contaminations occurred in biofilms and tank water and consisted mostly of *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Measures to reduce the risk of retrograde microbial contaminations of MDWS and a secure and lasting disinfection procedure were described.

KURZZUSAMMENFASSUNG

Es wurde untersucht, ob die fakultativ pathogenen Bakterien *Enterococcus faecalis*, *Pseudomonas aeruginosa* und *Escherichia coli* mobile Trinkwassersysteme retrograd kontaminieren können. Hierzu wurden regelmäßig an charakteristischen Stellen in praxisnahen Versuchsanlagen Wasser- und Biofilmproben entnommen und anhand geeigneter internationaler Normverfahren auf die fakultativ Pathogenen untersucht. Für den Nachweis von *Pseudomonas aeruginosa* fand zusätzlich zum Normverfahren die Fluoreszenz in situ Hybridisierung Anwendung. Jeder der fakultativ Pathogenen ist in der Lage mobile Trinkwassersysteme retrograd zu kontaminieren. Retrograde Kontaminationen konnten im Tankwasser und im Biofilm nachgewiesen werden, wobei *Pseudomonas aeruginosa* und *Enterococcus faecalis* häufiger nachgewiesen wurden als *Escherichia coli*. Abschließend wurden Empfehlungen für eine sichere und anhaltende Desinfektion sowie für Maßnahmen zur Verringerung des Risikos retrograder mikrobieller Kontaminationen gegeben.

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AOC	Easily assimilable organic carbon
AM	Arithmetic mean
APHA	Association of Port Health Authorities (United Kingdom)
Bs	Biofilm sampling section
CAC-filter	Compacted activated carbon filter
CC 20°C	Colony count determined at 20°C
CC 36°C	Colony count determined at 36°C
CDOC	Chromatographable DOC
CI	Confidence interval
DAPI	4’6-diamidino-2-phenylindole
DIN	“Deutsches Institut für Normung e.V.” standard
DOC	Dissolved organic carbon
DVGW	“Deutscher Verein des Gas- und Wasserfaches e.V.” standard
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
EN	European standard published by European Committee for Standardization
EPDM	Ethylene propylene diene monomer
FISH	Fluorescence in situ hybridization
FKM	Fluoroelastomer with fluor, perfluoroalkyl- and perfluoroalkoxy-groups
$HPC_{20°C}$	Heterotrophic plate count determined at 20°C
ISO	International standard published by International Standardization Organization
LC-OCD	Liquid chromatography with organic carbon detection
LC-UVD	Liquid chromatography with UV-detection
MDWS	Mobile drinking water system
OC	Organic carbon

P. aeruginosa	Pseudomonas aeruginosa
PE-Xb	Silane-coupled polyethylene
PE-Xc	Radiation-coupled polyethylene
PLC	Programmable logic controller
POM	Polyoxymethylene (Delrin® 100P BK602)
PTFE	Polytetrafluorethylene (Teflon®)
PVC	Polyvinyl chloride
Rig Con	Artificially microbiologically contaminated rig
Rig Ref	Reference rig
RP	Regrowth potential
STD	Standard deviation
TCC	Total cell count
TOC	Total organic carbon
TrinkwV	German drinking water directive, *ger*.: Trinkwasserverordnung
TS	Test series
TUHH	Technische Universität Hamburg-Harburg
US-EPA	United States Environmental Protection Agency
VBNC	Viable but nonculturable
WAM	Weighted arithmetic mean
WDP	Water distribution pipes
WHO	World health organisation

1 Introduction and objective of work

In a globalized world travel hygiene had become crucial. About 20 - 50 % of all travellers, representing 10 million people per year, acquire diarrhoea, the most common symptom of waterborne infections (WHO 2008).

According to WHO's 3rd edition of 'Guide to Hygiene and Sanitation in Aviation' (WHO 2009), US-EPA sampled drinking water from 327 aircraft in 2004 and found 15 % of the samples positive for total coliforms and 4,1 % of these positive for *Escherichia coli* (*E. coli*). The guide further refers to a study conducted by Health Canada in 2006 where most contaminations were found in water from lavatory faucets indicating rather a local contamination than a systemic water contamination. Additionally, the guide refers to a study conducted by the Association of Port Health Authorities (APHA) in 1999, where 850 water samples were taken from mains, bowsers and aircraft. 27 % of all samples were tested positive for *Pseudomonas aeruginosa* (*P. aeruginosa*), 7,8 % for total coliforms, 0,4 % for *E. coli* and 1,2 % for enterococci.

Beside aircraft, trains and ships, especially cruise ships, could serve as a vehicle of disease spread. In a review of 100 ship-associated outbreaks of infectious diseases between 1970 and 2000, water was identified as the transmission route in one fifth of all cases (WHO 2001).

Reports on water-borne infectious diseases associated with railways are rare, one publication documents the contamination of the drinking-water causing shigellosis (White 1976).

Under the assumption that contaminated water from the lavatory faucet originates from a local and not from a systemic contamination, the lavatory faucet has to be contaminated regularly from outside. Consequently, a retrograde contamination from the lavatory faucet into the mobile drinking water system (MDWS) becomes a certain risk factor. The retrograde microbial colonization of drinking water systems was already demonstrated in dental unit water systems where streptococci from oral origin were identified in the plumbing (Walker et al. 2000).

Regarding the findings of US-EPA, Health Canada, APHA, WHO (2001) and Walker et al. (2000) the question arises, how an artificial faecal contamination at the faucet of the lavatory assembly affects the microbial quality of a MDWS.

This thesis is based on a confidential industrial research and development project carried out at the institute of Water Resources and Water Supply (TUHH B-11) and at the DVGW-Forschungsstelle TUHH with Dr. Bernd Bendinger as project manager (Authors of confidential report: Moritz, J., Bendinger, B., 2011).

2 Concept

Two test rigs simulate each an independent MDWS. One represents a reference, the other one is artificially contaminated with facultative pathogenic bacteria at the faucet of the lavatory assembly after a biofilm has established. The artificial contamination at the faucet simulates a local contamination by unwashed hands touching the faucet after toilet usage. The artificial contamination contains *P. aeruginosa*, *E. coli* and *Enterococcus faecalis* (*E. faecalis*) as a representative for enterococci. The development of the microbial water quality and the biofilm formation in the water pipes is monitored for 22 weeks while the system is operated according to a representative consumption profile. The fill-up water quality is varied in terms of nutrient concentration and temperature, so that finally the occurrence of a retrograde contamination can be assessed regarding these two main factors.

3 Literature review

It is well known that traveling, especially international traveling, is associated with contracting and spread of infectious disease (Erdogan et al. 2010; Joseph et al. 2010; Marienau et al. 2010; Mangili and Gendreau 2005). However, reports on the microbiological water quality of mobile drinking water systems and their role as reservoir for pathogens are very limited, even though Dunott suggested already in 1878 that small-pox, malignant fever or syphilis could be contracted from the drinking water vessel of railways where it is “conveniently accessible under the spigot of the water cooler”.

3.1 Contamination sources for mobile drinking water systems

According to Freeman and Lock (1995) and WHO (2009) there are four critical points that could serve as a contamination source for MDWS:

1. **Contaminated fill-up water**

   The purchased water is already contaminated, e.g. by treatment failure, main break or cross-connection.

2. **Contaminated transfer points**

   Cross-contamination from improper handling and bad maintenance of e.g. water boats, water trucks, filling stations, hoses or the transfer connections themselves.

3. **Cross-connections**

   Direct connections to systems that do not contain potable water, e.g. automatic sprinkler systems, sewage systems or wash water systems. If the MDWS is pressurized with contaminated compressed air, the water can be contaminated as well.

4. **Retrograde contamination**

   Backflow from contaminated terminal consumption points like faucets in lavatories. Another possibility is the backgrowth of microorganisms against the flow direction.

3.2 Parameters affecting microbial growth

Even though drinking water is an oligotrophic environment it is suitable to promote microbial growth.

3.2.1 Biodegradable substances

One key-factor in promoting microbial growth is the concentration of biodegradable substances. Following van der Kooij (2003), the concentration of dissolved organic carbon (DOC) as energy source and substance for the formation and maintenance of biomass is the most relevant substance. Beside the DOC delivered with the bulk water, the origin of biodegradable organic carbon (BDOC) could be the material in contact with water (Speh et al. 1976; Colbourne and Brown 1979; Ellgas and Lee 1980; Frensch et al. 1987; Kilb et al. 2003). In order to maintain an unchlorinated water that does not promote the growth of bacteria, a concentration of easily assimilable organic carbon (AOC) below 10 µg/L has been derived as reference value (van der Kooij 1992). According to LeChevallier et al. (1991) the regrowth of coliform bacteria was reduced significantly in chlorinated supplies at AOC levels below 50 µg/L.

Besides organic carbon, ammonia could serve as substrate for microbial growth in treated water (van der Kooij 2003).

Waters from boreal regions contain typically a large amount of organic carbon (Vartiainen and Lilimatainen 1988). Miettinen et al. (1997) found that microbial growth in drinking water from boreal regions is highly regulated not only by organic carbon but also by the availability of phosphorus.

Miettinen et al. (1998) found that oxidizing agents like ozone, hydrogen peroxide or chlorine split organic compounds of high molecular weight to AOC, enabling the growth of heterotrophic bacteria.

3.2.2 Temperature

It is well known that an elevated temperature increases biological activity. In order to maintain microbiologically save water, cold drinking water should be constantly colder than 25°C (DIN EN 806-2) or above 60°C (DVGW W 551) for warm water at any consumption point. It has to be mentioned that even lower temperatures than 25°C could not prevent contaminations with pathogens, e.g. LeChevallier et al. reported in 1996 that temperatures above 15°C can enable the growth of *E. coli*.

3.2.3 Presence of sediments and corrosion products

Sediments consist of organic and inorganic material. In the case of organic sediments, e.g. from sloughed biofilm, it can serve as nutrient source (LeChevallier at al. 1987). Additionally, sediments or corrosion products can work like a protector against disinfectants (LeChevallier et al. 1990).

3.2.4 Hydraulic conditions

The flow velocity depends on the geometry of the drinking water system. Everything that is transported with the bulk water, especially bacteria, substrates and disinfectants, has an impact on the colonization of the water distribution system. If the velocity is high enough it will remove biofilm from the surface. In the case of chlorinated water residence time is a crucial parameter, since free chlorine concentration decreases with increasing time and provides the possibility of microbial growth (Lu et al. 1995).

3.2.5 Disinfection residual

In order to protect against pathogens and to prevent regrowth, most water supplies in North America and Europe maintain a disinfectant residual in the water throughout the entire distribution (Trussel 1999). The discovery of trihalomethane (THM) formation by chlorination (Rook 1977) led to restricted use of chlorine (van der Kooij 1999). Since disinfectants oxidize complex organic matter to AOC (Miettinen et al. 1998), the use of an insufficient concentration of disinfectant may support the growth of pathogens. Consequently, a sufficient disinfectant residual has to be maintained. The application of disinfectants in drinking water for the protection against pathogens should be limited to the cases where other measures fail (van der Kooij 2003).

3.3 Hygienic relevant microorganisms in water distribution systems

Pathogens known to be transmitted by drinking water are diverse. WHO's "Guidelines for Drinking-water quality" (WHO 2008) provides a list containing general information on pathogens and their significance in water supplies (Table 1).

Table 1: Water associated pathogens and their significance in water supplies, adapted from WHO (2008).

Pathogen	Health significance[1]	Persistence in water supplies[2]	Resistance to chlorine[3]	Relative infectivity[4]
Bacteria				
Burkholderia pseudomallei	High	May multiply	Low	Low
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate
E. coli - pathogenic[5]	High	Moderate	Low	Low
E. coli - enterohaemorrhagic	High	Moderate	Low	High
Legionella spp.	High	May multiply	Low	Moderate
Non-tuberculous mycobacteria	Low	May multiply	High	Low
P. aeruginosa[6]	Moderate	May multiply	Moderate	Low
Salmonella typhi	High	Moderate	Low	Low
Other salmonellae	High	May multiply	Low	Low
Shigella spp.	High	Short	Low	High
Vibrio cholerae	High	Short to long[7]	Low	Low
Yersinia enterocolitica	Moderate	Long	Low	Low
Viruses				
Adenovirus	Moderate	Long	Moderate	High
Enterovirus	High	Long	Moderate	High
Astroviruses	Moderate	Long	Moderate	High
Hepatitis A virus	High	Long	Moderate	High
Hepatitis E virus	High	Long	Moderate	High
Noroviruses	High	Long	Moderate	High
Sapoviruses	High	Long	Moderate	High
Rotaviruses	High	Long	Moderate	High
Protozoa				
Acanthamoeba spp.	High	May multiply	Low	High
Cryptosporidium parvum	High	Long	High	High
Cyclospora cayetanensis	High	Long	High	High
Entamoeba histolytica	High	Moderate	High	High
Giardia intestinalis	High	Moderate	High	High
Naegleria fowleri	High	May multiply[8]	Low	Moderate
Taxoplasma gondii	High	Long	High	High
Helminths				
Dracunculus medinensis	High	Moderate	Moderate	High
Schistosoma spp.	High	Short	Moderate	High

[1] Health significance relates to the severity of impact, including association with outbreaks.

[2] Detection period for infective stages in water at 20°C: short < 1 week; moderate 1 week - 1 month; long > 1 month.

[3] When the infective stage is freely suspended in water, treated at conventional doses and contact times and pH between 7 and 8. Low means 99 % inactivation at 20°C generally in < 1 min, moderate 1-30 min and high > 30 min. It should be noted that organisms that survive and grow in biofilms, such as *Legionella* and mycobacteria, will be protected from chlorination.

[4] From experiments with human volunteers, from epidemiological evidence and from animal studies. High means infective doses can be 1-10^2 organisms or particles, moderate 10^2-10^4 and low > 10^4.

[5] Includes enteropathogenic, enterotoxigenic and enteroinvasive.

[6] Main route of infection is by skin contact, but can infect immunsuppressed or cancer patients orally.

[7] Vibrio cholerae may persist for long periods in association with copepods and other aquatic organisms.

[8] In warm water.

When drinking water is exposed to more susceptible consumers, such as elderly, very young people or those with a suppressed immune defence system, further organisms than listed in Table 1 become relevant. Exner et al. (2007) provide an overview of drinking-water-associated pathogens relevant for health care facilities (Table 2).

Table 2: Water associated pathogens in health care facilities, adapted from Exner et al. (2007).

Pathogen
Legionella spp.
P. aeruginosa
Enterobacteriaceae (E. coli, Serratia spp.*, Klebsiella* spp.*, Enterobacter* spp.*)*
Acinetobacter spp.
Burkholderia cepacia
Stenotrophomonas maltophilia
Sphingomonas spp.
Ralstonia pickettii
Non-tuberculosus mycobacteria
Molds *(Aspergillus* spp.*, Fusarium* spp.*)*
Amoeba-associated bacteria *(Legionella anisa, Bosea massiliensis)*

3.4 Role of biofilms

Biofilms develop on all surfaces of drinking water systems, which are in contact with non-sterile water (Flemming 2011). Beside the presence of biofilms, they harbour 95 % of the bacteria while only 5 % are located in the water phase and commonly only water samples were analysed for quality control in water supplies (Flemming et al. 2002). Since microorganisms in biofilms are embedded in a matrix of extracellular polymeric substances (EPS) of their own origin their life differs much from planktonic microorganisms (Donlan 2002). According to Flemming and Wingender (2010) the biofilm matrix provides following benefits for its microorganisms:

1. Mechanically stable environment enabling the development of a synergistic microconsortium of microorganisms but without being a multicellular organism.
2. Sorption of organic carbon and inorganics in combination with extracellular enzymes make the biofilm to an external digestion system.
3. The matrix is a buffer-system for nutrients and water.
4. Exchange of genetic information by horizontal gene transfer.

5. Intercellular communication (quorum sensing)
6. Protection against disinfectants and antibiotics. Low concentrations of biocides can even enhance microbial activity.

Not only the microorganisms but also the humans benefit from biofilms. Biofilms play an important role in self-cleaning processes of contaminated sites. Additionally, biofilms in filters for drinking water treatment are used for purification (Keil et al. 2000; Gimbel et al. 2006) and as activated sludge in wastewater treatment (Wuertz et al. 2003). Establishment of drinking-water biofilms on materials employed for drinking water requires usually several weeks (Benölken 2010) to months (Wingender and Flemming 2004).

Total cell counts (TCC) range from 10^5 cells/cm^2 on copper and radiation-coupled polyethylene (PE-Xc) to a TCC of 10^7 to 10^8 cells/cm^2 on ethylene propylene diene monomer (EPDM). Heterotrophic plate count (HPC) varies between 10^1 to 10^3 cfu/cm^2 on copper and PE-Xc and between 10^6 to 10^7 cfu/cm^2 on EPDM. The culturable proportion (HPC) of the TCC varies between 0,01 % to a few percent (Benölken 2010). Wingender and Flemming (2004) found TCC values of 10^6 to 10^7 cells/cm^2 and HPC values of 10^3 to 10^5 on stainless steel, copper, PVC and PE coupons installed in biofilm reactors. They found no significant difference in the extent of biofilm formation between stainless steel, PVC and PE, whereas copper revealed a slightly higher TCC. The culturable proportion varied between 0,01 % to 2,63 %. Additionally, Wingender and Flemming (2004) investigated pipes cut out from different German drinking water biofilms and found a culturable proportion of 0,0004 % to 3,5 %. Low culturablity is considered to be typical for drinking water biofilms (Kalmbach et al. 1997).

Since biofilms contain living microorganisms, its growth is influenced by the same parameters already listed in chapter 3.2 and additionally by protozoa grazing the biofilm (Pedersen 1990).

Biofilms and health risks

When suitable conditions are provided all relevant water-associated pathogenic bacteria (chapter 3.3) are able to adhere to solid surfaces and form biofilm by multiplication and EPS production (Wingender and Flemming 2011a).

In contrast to bacteria, enteric viruses and parasitic protozoa are obligate parasites, i.e. they cannot multiply in biofilms of drinking water systems, they need their human or animal host. Similar to bacteria, viruses in biofilms are protected against disinfectants like chlorine (Quignon et al. 1997). The interaction of oocysts from *Cryptosporidium* spp. with drinking water biofilms is up to now quite unclear (Angles et al. 2007). However, biofilms seem to be a reservoir for *Cryptosporidium* oocysts and *Giardia* cysts (Howe et al. 2002).

Pathogens can be subdivided in pathogens from faecal and environmental origin. The faecal pathogens *Salmonella enterica*, *Shigella* spp., *Vibrio cholerae*, pathogenic *E. coli*, *Yersinia enterocolitica*, *Campylobacter* spp. and *Helicobacter pylori* carry the potential of becoming a member of biofilms (Wingender 2011b). Presence of microorganisms from faecal origin is commonly assessed via detection of coliforms/*E. coli* (DIN EN ISO 9308-1) and often additionally with the detection of Enterococci (DIN EN ISO 7899-2). The environmental opportunistic pathogens *Aeromonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, *Legionella* spp., *Mycobacteria* spp. and *P. aeruginosa* can persist or even multiply in drinking-water biofilms. Their infective dose for healthy individuals is in general between 10^4 and > 10^{10} (Rusin et al. 1997).

3.5 Examples for retrograde contaminations in water distribution systems

The term “retrograde” contamination regarding drinking water systems is used in two ways. One way is the contamination of a terminal consumption point from outside, e.g. a contamination of a faucet by a patient (Reuter et al. 2002). In this case “retrograde” describes the path from patient to consumption point. The second way is the contamination of a terminal consumption point followed by a backgrowth or backflow into the drinking water system. In this case “retrograde” represents the path into the drinking water system. The latter definition is the one applied to the present work. The retrograde microbiological contamination is only documented for dental care units. In the plumbing of dental facilities streptococci were identified, which are known to be from oral origin (Walker et al. 2000).

4 Materials and methods

4.1 Setup of test rigs

Two test rigs of identical construction were installed at Technical University Hamburg-Harburg (TUHH). One rig was operated as reference (Rig Ref) whereas the other system was artificially contaminated with a suspension of bacteria (Rig Con).

Each test rig simulated an independent MDWS, which basically consisted of an insulated and temperature-controlled tank, stainless steel pipes to fill and drain the MDWS and to connect the tank with the consumption points in the lavatory assembly and the galley assembly (Figure 1). The lavatory assembly was equipped with a heater and a mixing unit to produce warm water for the faucet. Another outlet next to the faucet simulated the consumption of a toilet. The galley assembly was equipped with a compacted activated carbon filter (CAC-filter), a faucet and an outlet to simulate the consumption of a coffee maker. In order to distinguish between the faucets of the lavatory and the galley assembly, the faucet in the galley assembly was named spigot. The entire MDWS fulfilled the requirement of self-venting and self-draining and therefore all drinking water pipes (WDP) were sloped in an angle of 3°. The CAC-filter was equipped with a bypass, which ensured drainage of the galley without backflushing the CAC-filter. A unidirectional restrictor valve in the bypass prevented the section downstream the CAC-filter from a contamination with non-filtered water. The entire set-up was controlled by a programmable logic controller (PLC).

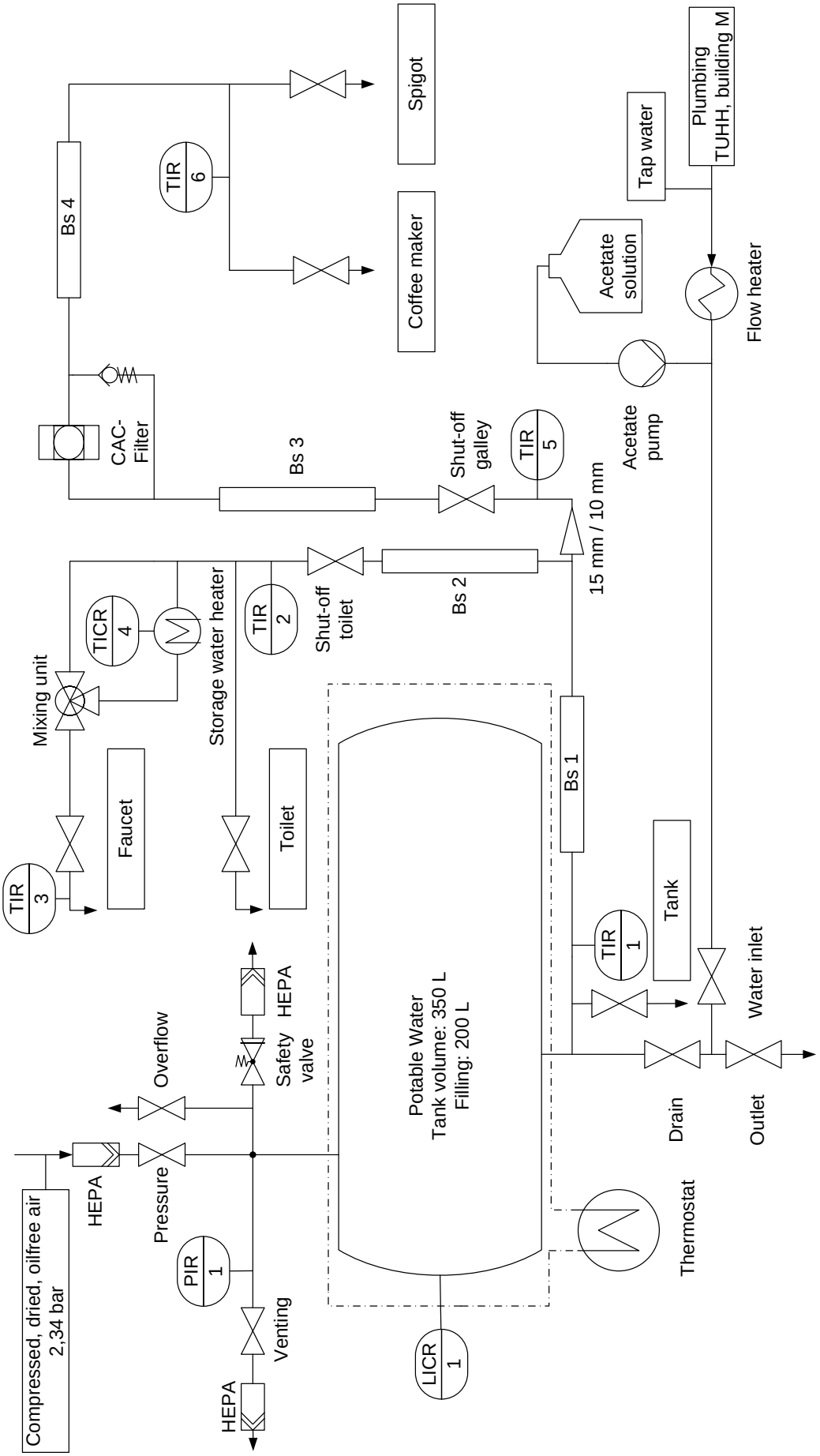

Figure 1: Schematic flow chart of a test rig

The following subunits of the MDWS are listed according to the main flow direction of the water.

4.1.1 Flow heater

For test series on elevated temperature level (chapter 4.3) the fill-up water was tempered to 36°C during the entire fill-up phase. During test series at room temperature the flow heater was switched off. For thermal disinfection the fill-up water was tempered to approximately 80°C during entire fill-up phase to achieve a temperature of at least 70°C at the most distant point of the MDWS (coffee maker).

4.1.2 Acetate pump

According to the desired concentration (Table 7) heat sterilized Na-acetate solution was pumped continuously into the fill-up water during the entire fill-up phase.

4.1.3 Tank

The tank had a volume of 350 L and was made of carbon fibre with an inner coating made of polypropylene. In order to control the temperature of the tank water, the outer surface was wrapped with water perfused tubes. The tubing was connected within a closed circuit to a thermostat, which circulated and heated or cooled the water for the tubing. The tank and the tubing were additionally wrapped by a thermal insulating foam. In order to control fill-up and drainage, the tank was equipped with a level meter, which was connected to the PLC. The valves “Water inlet”, “Outlet”, “Drain”, “Pressure” and “Venting” were controlled by the PLC according to the consumption profile (chapter 4.3). Due to the applied pressure (chapter 4.3) the water flowed from the tank through the first biofilm sampling array (Bs 1) to the lavatory assembly and the galley assembly.

4.1.4 Lavatory assembly

The lavatory assembly consisted of a biofilm sampling section (Bs 2), a manual ball valve (Shut off lavatory), an outlet in order to simulate the consumption of a toilet (toilet), a storage water heater, a mixing unit and another outlet in order to simulate the consumption of a lavatory faucet. The toilet and the mixing unit were controlled by the PLC according to the consumption profile (chapter 4.3). The temperature of the water in the storage water heater was set to 50°C and was as well controlled by the PLC. In order to simulate a consumption of warm water at the faucet the valves for hot and cold water were opened simultaneously during consumption at the faucet. The temperature of the warm water at the faucet is a product of a 1:1 mixture of cold water and hot water from the storage water heater.

The faucet of Rig Con was the outlet of the system that was microbiologically contaminated once a week. The weekly contamination was started after a phase of 6 weeks of operation enabling an establishment of an autochthonous biofilm.

4.1.5 Galley assembly

The galley assembly consisted of two biofilm sampling sections, the first section (Bs 3) was installed before and the second section (Bs 4) was installed downstream the CAC-filter. Activated-carbon filtration is usually used to improve taste, odour and colour of water. Consequently, the CAC-filter will have an impact on the microbial quality of the water. The galley assembly was further equipped with a manual ball valve (Shut off galley) and a CAC-filter bypass with an unidirectional restrictor valve in order to prevent the section downstream the CAC-filter from a contamination with non-filtered water. Two outlets were installed in the galley assembly. The first outlet simulated the consumption of a coffee maker (coffee maker) and the second outlet the consumption of a spigot. The coffee maker and the spigot were controlled according to the consumption profile (chapter 4.3) by the PLC.

4.1.6 Water distribution pipes

The water distribution pipes (WDP) were installed in the test rigs with a slope of 3° to ensure self-draining of the MDWS. Length, inner diameter and the type of material in contact with water are listed in Table 3. All WDP were not thermally insulated.

Table 3: Length, inner diameter and type of material in contact with water of pipe sections

Pipe section	**Length**	**Inner diameter**	**Material in contact with water**
-	[m]	[mm]	-
Plumbing TUHH ←→ Flow heater	13,50	15	PE-Xb
Flow heater ←→ acetate dosage	2,19	15	Stainless steel
Acetate dosage ←→ standpipe	0,23	15	Stainless steel
Standpipe	1,40	15	Stainless steel
Tank ←→ Bs 1	1,30	15	Stainless steel
Bs 1	6,70	15	Stainless steel
Bs 1 ←→ T-joint “assemblies”	7,85	15	Stainless steel
Lavatory assembly			
T-joint “assemblies” ←→ Bs 2	0,50	15	Stainless steel
Bs 2	6,70	15	Stainless steel
Bs 2 ←→ toilet	0,80	15	Stainless steel
Bs 2 ←→ T-joint “storage water heater/ mixing unit”	0,28	15	Stainless steel
T-joint “storage water heater/ mixing unit” ←→ storage water heater	0,17	15	Stainless steel
Storage water heater ←→ mixing unit	0,30	5	PTFE
T-joint “storage water heater/ mixing unit” ←→ mixing unit	0,40	5	PTFE
Mixing unit ←→ faucet	0,54	5	PTFE
Galley assembly			
T-joint “assemblies” ←→ shut off galley	0,10	10	Stainless steel
Shut off galley ←→ Bs 3	0,41	10	Stainless steel
Bs 3	6,70	10	Stainless steel
Bs 3 ←→ CAC-filter	0,54	10	Stainless steel
Bs 3 ←→ unidirectional restrictor valve	0,45	10	Stainless steel
CAC-filter ←→ Bs 4	0,60	10	Stainless steel
Bs 4	6,70	10	Stainless steel
Bs 4 ←→ spigot	0,78	10	Stainless steel
Bs 4 ←→ coffee maker	0,92	10	Stainless steel

PE-Xb Silane coupled polyethylene

PTFE Polytetrafluorethylene (Teflon®)

4.1.7 Valves

The valves listed in Table 4 are designed for applications in drinking water systems. The PLC-controlled valves were used to operate the MDWS according to the consumption profile (chapter 4.3). The manually controlled valves were solely necessary for sampling (tank) and for disinfection (chapter 4.4).

Table 4: Valves in contact with water

	Name	**Type**	**Main material in contact with water**	**Sealing in contact with water**
PLC controlled	Water inlet	Solenoid valve	Stainless steel	FKM
	Outlet	Electrical ball valve	Stainless steel	PTFE
	Drain	Pneumatic ball valve	Stainless steel	PTFE
	Lavatory assembly			
	Toilet	Solenoid valve	Stainless steel	FKM
	Mixing unit	Solenoid valve	POM	EPDM
	Galley assembly			
	Spigot	Solenoid valve	Stainless steel	FKM
	Coffee maker	Solenoid valve	Stainless steel	FKM
Self controlled	**Galley assembly**			
	Unidirectional restrictor	Spring-loaded check-valve	Stainless steel	Silicone
Manually controlled	Tank	Manual ball valve	Stainless steel	PTFE
	Overflow	Manual ball valve	Stainless steel	PTFE
	Lavatory assembly			
	Shut off lavatory	Manual ball valve	Stainless steel	PTFE
	Galley assembly			
	Shut off galley	Manual ball valve	Stainless steel	PTFE

FKM Fluoroelastomer with fluor, perfluoroalkyl- and perfluoroalkoxy-groups
PTFE Polytetrafluorethylene Teflon®
POM Polyoxymethylene (Delrin® 100P BK602)
EPDM Ethylene propylene diene monomer

The valves from Table 5 were used to control the pressure of the entire MDWS according to the consumption profile (chapter 4.3).

Table 5: Valves in contact with pressurized air

	Name	**Type**
PLC controlled	Pressure	Solenoid valve
	Venting	Solenoid valve
Self controlled	Safety valve	Pressure safety valve

4.2 Artificial contamination

Contamination suspension: The suspension was a gel made of agar (3 g/L) and contained the three facultative pathogenic bacteria *P. aeruginosa*, *E. faecalis* and *E. coli*, each of them in a concentration of approximately 1 x 10^9 cfu/mL.

P. aeruginosa AdS	Aquatic Microbiology, University of Duisburg-Essen, Germany
E. faecalis DSM 20478	Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
E. coli DSM 30083	Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Cultivation: The bacteria were cultivated for 16 h in LB-Medium at 36°C. LB-Medium was prepared according to the protocol listed in 'Molecular cloning: a laboratory manual' (Maniatis et al. 1982). Erlenmeyer flasks containing the bacteria and LB-Medium were shaken at 140 rpm (Weiss Gallenkamp, Loughborough, United Kingdom) during incubation. After incubation, bacteria were washed twice by centrifugation (5000 g, 2 min) and resuspension in sterile tap water. Subsequently, the bacteria were suspended in the gel made of agar.

Contamination procedure: After six weeks of biofilm establishment with desired operating conditions (chapter 4.3), the faucet aerator of the lavatory faucet was artificially contaminated by dipping it into the contamination suspension (Figure 2). The contamination was conducted once a week representing a contamination every 560th usage of the faucet. The faucet aerator was immerged for approximately 20 min during subphase 11 of trip two but prior to the daily drainage of the system (Table 6). The removal of the contamination suspension prior to the drainage ensured that the suspension was not sucked into the MDWS.

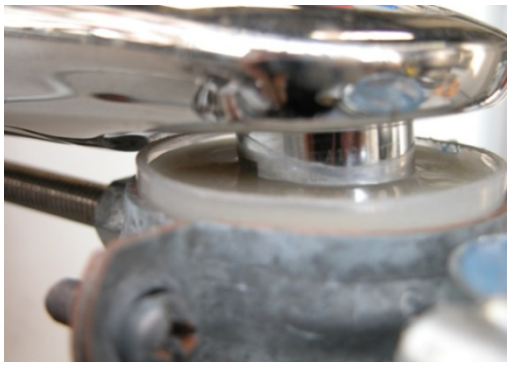

Figure 2: Application of the artificial contamination at the faucet

In contrast to the above described procedure the contamination in TS 1 was conducted only once during the drainage of the MDWS after six weeks of biofilm establishment. Due to the vacuum during drainage at the lavatory faucet the contamination suspension was sucked into the MDWS. Since the artificial contamination should simulate a scenario with unwashed hands touching the faucet after toilet usage it was concluded that the active suction from a reservoir is not realistic.

4.3 Operating conditions

The entire investigation was subdivided into seven test series. Each of them was characterised by certain boundary conditions (Table 7). Some boundary conditions were repeated in a new test series because TS 1, TS 2 and TS 4 were aborted because of inadequate contamination procedure or failed disinfection. Prior to and after each test series, Rig Ref and Rig Con were disinfected (chapter 4.4). An entire test series lasted 22 weeks and the first artificial contamination was applied at the faucet of Rig Con after six weeks of biofilm establishment. The biofilm establishment was necessary for two reasons. Firstly, it enabled the development of a native biofilm with autochthonous bacteria and secondly, it enabled the verification of the disinfection procedure under close to practice conditions. As both rigs were filled daily with approximately 200 L of tap water, followed by a consumption profile and finally drained, it is assumed that the simulation was characteristic for medium haul journeys (Table 6). During the fill-up the water was conditioned according to the desired boundary conditions (Table 7). In order to distribute the water from the tank to the consumption points, the system was pressurized after complete fill-up to 2,3 bar with dry, sterile and oil free air. Sterility of air was provided by an air-filter with a pore size of 0,01 µm.

4.3.1 Consumption profile

During the simulated trips the PLC-controlled valves were opened according to the consumption profile listed in Table 6.

Table 6: Consumption profile

Phase		Duration [min]	Toilet assembly				Total	Galley assembly				Total
			Toilet (0,2 L per activity)		Faucet (0,308 L per activity)			Spigot (0,5 L per activity)		Coffee maker (2 L per activity)		
			Activities	Vol. [L]	Activities	Vol. [L]	Vol. [L]	Activities	Vol. [L]	Activities	Vol. [L]	Vol. [L]
Non-operated		290	-	-	-	-	-	-	-	-	-	-
Fill-up		20	-	-	-	-	-	-	-	-	-	-
Trip 1	Subphase 1	30	-	-	-	-	-	-	-	-	-	-
	Subphase 2	15	3	0,6	3	0,924	1,524	-	-	-	-	-
	Subphase 3	120	17	3,4	17	5,236	8,636	2	1,0	2	4	5,0
	Subphase 4	15	8	1,6	8	2,464	4,064	2	1,0	2	4	5,0
	Subphase 5	120	24	4,8	24	7,392	12,192	-	-	-	-	-
	Subphase 6	15	8	1,6	8	2,464	4,064	-	-	-	-	-
	Subphase 7	60	12	2,4	12	3,696	6,096	-	-	-	-	-
	Subphase 8	10	3	0,6	3	0,924	1,524	-	-	-	-	-
	Subphase 9	60	-	-	-	-	-	3	1,5	3	6	7,5
	Subphase 10	15	5	1,0	5	1,540	2,540	-	-	-	-	-
	Subphase 11	30	-	-	-	-	-	-	-	-	-	-
	Total trip	490	80	16,0	80	24,640	40,640	7	3,5	7	14	17,5
											Total volume trip 1	**58,14**
Turn-around		120	-	-	-	-	-	-	-	-	-	-
Trip 2 (identical to trip 1)		490									**Total volume trip 2**	**58,14**
Drainage		30	-	-	-	-	-	-	-	-	-	-
Total		24 h	160	32,0	160	49,280	81,280	14	7,0	14	28	35,0
											Total Volume	**116,28**

4.3.2 Boundary conditions

Rig Ref and Rig Con were operated according to the boundary conditions listed in Table 7. TS 1 was aborted due to a non-realistic artificial contamination (chapter 4.2). Since contaminants were detected during the biofilm establishment, TS 2 and TS 4 were aborted. The occurrence of contaminants previous to the first artificial contamination must have been due to failed disinfection.

Table 7: Boundary conditions of test series

Test series	Aborted	Duration	Boundary condition		
			T [°C]	Na-acetate dosage [µg/ L]	
				Rig Ref	Rig Con
TS 1	Yes, due to inadequate contamination	12/2008 – 05/2009	20	0	100
TS 2	Yes, due to failed disinfection	05/2009 – 07/2009	36	0	100
TS 3	No	07/2009 – 12/2009	36	0	100
TS 4	Yes, due to failed disinfection	12/2009 – 03/2010	20	0	100
TS 5	No	03/2010 – 08/2010	20	0	100
TS 6	No	08/2010 – 01/2011	20	100	300
TS 7	No	01/2011 – 07/2011	36	100	300

The conducted test series allow the assessment of three impact factors:

1. Impact of artificial contamination (chapter 4.3.2.1)
2. Impact of acetate dosage (chapter 4.3.2.2)
3. Impact of operating temperature (chapter 4.3.2.3)

4.3.2.1 Impact of artificial contamination

In order to assess the impact of the artificial contamination, results from Rig Ref and Rig Con have to be compared. Consequently, operating temperature and acetate dosage have to be the same. Table 8 contains the combinations of comparable test series. Results of the impact of the artificial contamination can be found in chapter 5.3.

Table 8: Test series to assess the impact of the artificial contamination

Assessment of …	**Boundary condition**		**Test series**	
	T [°C]	**Na-acetate dosage [µg/L]**	**Rig Ref**	**Rig Con**
… impact of artificial contamination (chapter 5.3)	20	100	TS 6	TS 5
	36	100	TS 7	TS 3

4.3.2.2 Impact of acetate dosage

The impact of acetate dosage can be assessed in two ways:

- Comparison of results from only one MDWS, either Rig Ref or Rig Con, for those results that depend on the artificial contamination.
- Comparison of results from both MDWS, for those results that do not depend on the artificial contamination.

Table 9 contains the test series that provide assessment of the impact of acetate dosage. Results can be found in chapter 5.4. Rig Ref was operated with identical boundary conditions during TS 1 and TS 5 offering the possibility to check for reproducibility (chapter 5.2).

Table 9: Test series to assess the impact of acetate dosage

Assessment of …	**Boundary condition**		**Test series**	
	T [°C]	**Na-acetate dosage [µg/L]**	**Rig Ref**	**Rig Con**
… impact of acetate dosage (chapter 5.4)	20	0	TS 1, TS 5	-
		100	TS 6	TS 5
		300	-	TS 6
	36	0	TS 3	-
		100	TS 7	TS 3
		300	-	TS 7

4.3.2.3 Impact of operating temperature

Identical to the assessment of the impact of acetate dosage, the assessment of the impact of operating temperature can be achieved in two ways:

- Comparison of results from only one MDWS, either Rig Ref or Rig Con, for those results that depend on the artificial contamination.
- Comparison of results from both MDWS, for those results that do not depend on the artificial contamination.

Table 10 contains the test series that provide assessment of the impact of operating temperature. Results can be found in chapter 5.5.

Table 10: Test series to assess the impact of operating temperature

Assessment of …	**Boundary condition**		**Test series**	
	T [°C]	**Na-acetate dosage [µg/L]**	**Rig Ref**	**Rig Con**
… impact of temperature (chapter 5.5)	20	0	TS 1, TS 5	-
	36		TS 3	-
	20	100	TS 6	TS 5
	36		TS 7	TS 3
	20	300	-	TS 6
	36		-	TS 7

4.4 Disinfection

Previous to any disinfection of both test rigs the used CAC-filter had been removed, autoclaved and disposed. After disinfection a new CAC-filter had been mounted.

In order to ensure a secure disinfection of the contaminated faucet of Rig Con the aerator had been dismounted after the disinfection of any TS and was replaced before the disinfection for any new TS. The metallic fixation of the aerator was disinfected thermally with a Bunsen burner.

Three types of disinfection procedures were applied:

1. Two-step chemical disinfection with hydrogen peroxide

The two-step chemical disinfection was conducted with hydrogen peroxide (Herlisil, Feldmann Chemie, Inning, Germany). The first step consisted of filling the entire MDWS with disinfection solution with a hydrogen peroxide concentration of 500 ppm. After fill-up the system had been pressurized and all outlets had been flushed with at least 10 L of disinfection solution. Afterwards the valves “Shut-off toilet” and “Shut-off galley” were closed in order to maintain disinfection solution at the terminal consumption points during depressurization and subsequent fill-up. After final fill-up, the system was left unoperated for a holding time of 1 h allowing the disinfectant to react. When holding time had been expired, all outlets were flushed again with at least 10 L disinfection solution. The system was drained afterwards.

The procedure for the second step was a repetition of the first step with the deviation of a reduced holding time of 0,5 h.

After disinfection the MDWS had been rinsed twice with tap water.

2. Single-step chemical disinfection with sodium hypochlorite

The procedure for the single step disinfection is in general the same as described for the first step of the two-step chemical disinfection. In deviation from the two-step disinfection the disinfectant had been changed to sodium hypochlorite (Carl Roth, Karlsruhe, Germany) with a concentration of 100 ppm (free chlorine). With respect to the omitted second disinfection step, the holding time had been increased to 4 h and the volume for flushing each outlet had been doubled to at least 20 L of disinfection solution.

After disinfection the MDWS had been rinsed twice with tap water.

3. CTC-disinfection - Chemical, thermal, chemical disinfection

The CTC-disinfection consisted of a single-step chemical disinfection with hypochlorite according to disinfection procedure 2 and was followed by a thermal disinfection with hot tap water. During the thermal disinfection the hot tap water ran through the MDWS, so that the most distant or coldest point (coffee maker) was treated with at least 70°C for at least 3 min according to DVGW W 551. After thermal disinfection the systems were disinfected again with a single-step chemical disinfection according to disinfection procedure 2.

4.5 Sampling

Water sampling: Water samples were taken weekly from Rig Ref and Rig Con (Figure 3). Rig Ref was sampled solely at the sampling point of the tank. In order to investigate the development of the retrograde contamination Rig Con was sampled at the tank, spigot and faucet. Samples from the tank were taken manually during subphase 10 from the second trip (Table 6). Each sample from the spigot was a mixture of water samples of two simulated activities during subphase 9 of trip two (Table 6). Each water sample from the faucet was a mixture of water samples of three simulated activities during subphase 10 of trip two (Table 6). In order to assess the water quality of the stored water, the tank sampling point was disinfected thermally and flushed until temperature constancy according to DIN EN ISO 19458, case “a” (assessment of water quality in the drinking water installation). In contrast to the tank sampling point, the faucet and the spigot were not disinfected or flushed prior to or after sampling according to DIN EN ISO 19458, case “c”. This procedure enables the assessment of the water qualities, which were delivered by the faucet and the spigot during the entire time of operation (assessment of water quality as it is used by the consumer). In order to investigate a retrograde contamination it is important that the microbiology of the faucet remains undisturbed.

In order to characterise the fill-up water, samples were taken from the tank during the fill-up phase (chapter 4.3, Table 6).

Biofilm sampling: The biofilm sections Bs 1, Bs 2, Bs 3 und Bs 4 were sampled approximately after 2, 5, 10, 14, 18 and 22 weeks of operation during the non-operated phase of the consumption profile (Figure 3, Table 6).

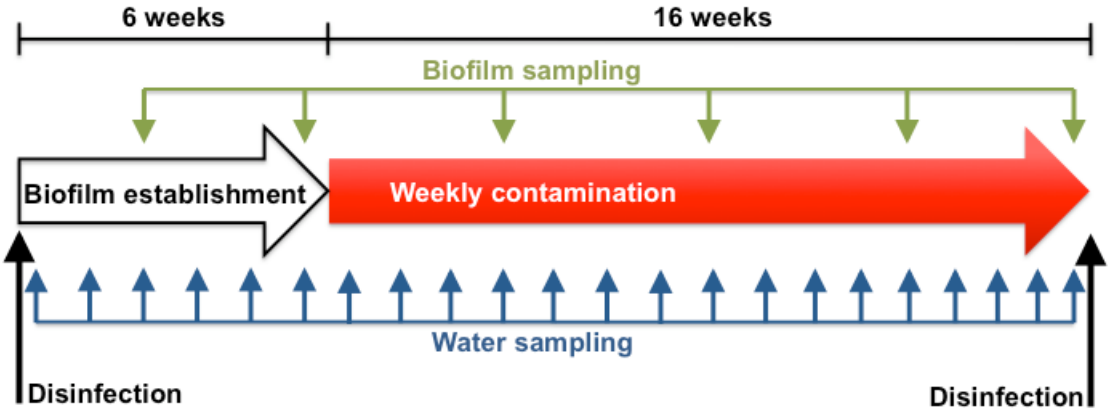

Figure 3: Flowchart of an entire test series

At any sampling a pipe segment with a length of one meter was dismounted from each of the biofilm sampling sections (chapter 4.1, Figure 1). The biofilm from the inner surface was removed mechanically in three steps. Previous to the first step the pipes were filled to one-fourth with sterile ceramic beads (SAZ ER 120 S, 0,3 - 0,4 mm, Mühlmeier, Bärnau, Germany). Each removal step consisted of following four substeps:

1. Filling of pipes to one half with sterile water
2. Shaking at 250 rpm in axial direction (SM 25 Edmund Bühler GmbH, Hechingen, Germany)
3. Rotation of pipes every 2 min by 90° over their radial axis. The pipes were turned in total three times so that the ceramic beads scratched off the biofilm from the entire inner surface.
4. Decanting of biofilm suspension into a sterile flask for further analysis

After the third removal step pipes were rinsed with sterile water in order to remove ceramic beads. Subsequently, each pipe segment was mounted again in its sampling section.

4.6 Chemical and physical methods

pH-value: The pH-value was determined according to DIN 19261.

Electrical conductivity: The electrical conductivity was determined according to DIN EN 27888 (ISO 7888:1985).

Ions: The concentrations of sodium, potassium, calcium and magnesium were determined according to DIN EN ISO 11885. Copper was determined according to DIN EN ISO 17294-2. The concentrations of chloride, sulphate, phosphate and nitrate were determined according to DIN EN ISO 10304-1. Nitrite was determined according to DIN EN 26777 (ISO 6777:1984) and ammonium was determined according to DIN 38406.

Acidity and Alkalinity: The acidity was determined by HCl-titration until the pH-value of 4,3 ($acidity_{4,3}$) and the alkalinity was determined by NaOH-titration until the pH-value of 8,2 ($alkalinity_{8,2}$) according to DIN 38409-7.

Spectral adsorption coefficient at 436 nm (SAC_{436}): The adsorption coefficient at 436 nm was determined according to DIN EN ISO 7887. The filter pore size of the filter (polycarbonate track etch membrane, Whatman, Maidstone, United Kingdom) for the sample preparation for SAC_{436} measurement was 0,4 µm. This was 0,05 µm smaller than the mandatory filter pore size of DIN EN ISO 7887. It was assumed that this difference had no relevant impact on the determined SAC_{436} since tap water contains no particles.

Spectral adsorption coefficient at 254 nm (SAC_{254}): The adsorption coefficient at 254 nm was determined according to DIN 38404-3. The filter pore size of the filter (polycarbonate track etch membrane, Whatman, Maidstone, United Kingdom) for the sample preparation for SAC_{254} measurement was 0,4 µm. This was 0,05 µm smaller than the mandatory filter pore size of DIN 38404-3. It was assumed that this difference had no relevant impact on the determined SAC_{254} since tap water contains no particles.

Dissolved oxygen concentration (O_2-concentration): The concentration of dissolved oxygen was determined according to DIN EN 25814 (ISO 5814:1990).

Total organic carbon (TOC) and dissolved organic carbon (DOC): The concentrations of TOC and DOC were determined according to DIN EN 1484. The filter pore size of the filter (polycarbonate track etch membrane, Whatman, Maidstone, United Kingdom) for the sample preparation for DOC measurement was 0,4 µm. This is 0,05 µm smaller than the mandatory filter pore size of DIN EN 1484. It is assumed that this difference had no relevant impact on the determined concentration of DOC.

Liquid chromatography with OC- and UV-detection (LC-OCD, LC-UVD): Fractionation and quantification of chromatographable DOC (CDOC) was performed according to Huber and Frimmel (1996). Following CDOC-fractions could be identified and quantified:

- polysaccharides and colloids (PS/Colloids)
- humic substances (HS)
- building blocks (BB)
- low molecular weight compounds (LMWC)
- amphiphilic and neutral compounds (AC and NC)

The sum of the fractions is the CDOC. Beside the measurement of CDOC, the DOC_{UV} was determined. It differs from the DOC by the oxidation method. The DOC was thermally oxidized and the DOC_{UV} was oxidized by intense UV radiation with a wavelength of 254 nm.

4.7 Microbiological methods

Total cell count (TCC): The TCC was determined microscopically. Therefore, 1 to 10 mL of biofilm suspension were filtered through a 0,2 µm membrane filter (polycarbonate track etch membrane, Whatman, Maidstone, United Kingdom). The bacteria on a filter were counted after staining with the DNA-specific stain 4'6-diamidino-2-phenylindole (DAPI) according to Porter and Feig (1980).

Heterotrophic plate count (HPC): The HPC was determined after 14 d of incubation at 20°C according to Reasoner and Geldreich (1985).

Colony count determined at 20°C (CC 20°C): The CC 20°C was determined according to the German drinking water directive of 1990 (TrinkwV 1990).

Colony count determined at 36°C (CC 36°C): The CC 36°C was determined according to TrinkwV 1990.

Colony counts of *P. aeruginosa*: The colony counts of *P. aeruginosa* were determined on a selective medium according to DIN EN ISO 16266.

Colony counts of *E. faecalis*: The colony counts of *E. faecalis* were determined on a selective medium according to DIN EN ISO 7899-2.

Colony counts of *E. coli*: The colony counts of *E. coli* were determined on a selective medium according to DIN EN ISO 9308-1.

Fluorescence in situ hybridization (FISH): For cultivation-independent detection of *P. aeruginosa* the FISH-probe Psae16S-182 was applied at 46°C. In order to create desired stringency or rather probe-selectivity, the hybridization buffer contained 40% formamide according to Wellinghausen et al. (2005). Additionally, for each sample the probe NON338 (NONEUB) was used as a control for non-specific binding (Wallner et al. 1993). Parallel to any FISH a fixed overnight culture of *P. aeruginosa* served as a positive control for the hybridization process. In general, FISH was performed according

to the method developed by Amann et al. (1990). For detection of *P. aeruginosa* the hybridization conditions described by Wellinghausen et al. (2005) and for detection of non-specific binding the conditions of Wallner et al. (1993) were applied.

Regrowth potential (RP): The RP is the maximal concentration of autochthonous bacteria that develop in the water sample at 15°C during the analysis period of six weeks. The concentration of autochthonous bacteria was determined according to the HPC.

Easily assimilable organic carbon (AOC): The concentration of AOC was determined according to van der Kooij et al. (1982 a).

5 Results and discussions

5.1 Characterization of fill-up water and water temperature inside the rigs

Any of the tested parameters for the fill-up water characterization were within the limiting values of TrinkwV 2001 if regulated.

As none of the contaminants was detected in Rig Ref, neither in water nor in biofilm samples, it had been demonstrated that the fill-up water was free of *P. aeruginosa*, *E. faecalis* and *E. coli* at any time.

In general the fill-up water was characterized by the HPC, CC 20°C, CC 36°C, electrical conductivity, alkalinity, acidity, SAC_{436}, SAC_{254}, concentrations of cations and anions (chapter 5.1.1). Additionally the oxygen concentrations and pH-values of the fill-up water are given for the two operating temperatures.

The parameters RP, AOC, TOC, DOC, DOC_{UV}, CDOC and its fractions consisting of PS/colloids, HS, BB, LMWC, AC and NC were used to characterize the fill-up water with different dosages of acetate (chapter 5.1.2).

The water temperatures at certain points inside the test rigs (tank, toilet assembly, faucet, galley assembly and spigot) are given in Table 18 (chapter 5.1.3).

5.1.1 Parameters characterizing the fill-up water

Parameters for the characterization of the fill-up water can be taken from Table 11 to Table 15.

Table 11: HPC, CC 20°C and CC 36°C of fill-up water. Values are given as weighted arithmetic mean (WAM) and 95 % confidence interval (CI)

HPC	CC 20°C	CC 36°C
[cfu/mL]	[cfu/mL]	[cfu/mL]
592 ± 11	< 1	< 1

Table 12: Electrical conductivity, alkalinity$_{8,2}$, aciditiy$_{4,3}$, SAC$_{436}$ and SAC$_{254}$ of fill-up water. Values are given as arithmetic mean (AM) and standard deviation (STD)

El. conductivity	Alkalinity$_{8,2}$	Acidity$_{4,3}$	SAC$_{436}$	SAC$_{254}$
[mS/cm]	[mmol/L]	[mmol/L]	[1/m]	[1/m]
244 ± 1	0,04 ± 0,01	2,00 ± 0,02	0,03 ± 0,01	1,48 ± 0,03

Table 13: Concentrations of cations in fill-up water. Values are given as AM and STD.

Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	Cu^{2+}
[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
8,42 ± 0,83	1,36 ± 1,17	37,64 ± 1,05	2,20 ± 0,04	0,06 ± 0,03	0,07 ± 0,03

Table 14: O_2-concentration and pH-value of fill-up water

T	O_2-concentration	pH-value
[°C]	[mg/L]	[-]
20	11,43 ± 0,06	7,94 ± 0,03
36	8,94 ± 1,20	7,88 ± 0,08

Table 15: Concentrations of anions in fill-up water. Values are given as AM and STD.

NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Cl^-
[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
< 0,01	< 0,5	< 0,5	10,81 ± 0,77	10,10 ± 0,29

5.1.2 Parameters of fill-up water after dosing of acetate

Parameters characterizing the fill-up water with different dosages of acetate can be taken from Table 16 and Table 17. The parameters RP, TOC, DOC and AOC increased with dosage of Na-acetate.

Table 16: RP, TOC, DOC and AOC of fill-up water. Values for RP are given as AM and 95 % CI. Values for TOC, DOC and AOC are given as AM and STD.

Na-acetate dosage	RP	TOC	DOC	AOC
[µg/L]	[cfu/mL]	[mg/L]	[mg/L]	[µg ac-C eq/L]
0	2,0E+05 ± 9,7E+04	0,72 ± 0,06	0,78 ± 0,07	5 ± 7
100	4,0E+05 ± 1,6E+05	0,93 ± 0,11	0,89 ± 0,07	15 ± 10
300	1,1E+06 ± 8,2E+05	1,00 ± 0,10	0,99 ± 0,05	52 ± 27

Table 17: DOC_{UV}, CDOC, PS/Colloids, HS, BB, LMWC, AC and NC of fill-up water. Values are given as AM and STD.

Na-acetate dosage	LC-OCD						
	DOC_{UV}	CDOC	PS/Colloids	HS	BB	LMWC	AC and NC
[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
0	899 ± 346	573 ± 98	4 ± 3	269 ± 87	157 ± 20	16 ± 20	127 ± 41
100	849 ± 155	640 ± 110	5 ± 5	254 ± 60	169 ± 44	63 ± 54	150 ± 54
300	871 ± 63	649 ± 77	4 ± 4	243 ± 24	152 ± 27	104 ± 35	145 ± 52

5.1.3 Water temperatures in the test rigs

Water temperatures from the test series TS 1, TS 3, TS 5, TS 6 and TS 7 can be taken from Table 18. There was no temperature difference between Rig Ref and Rig Con.

Since the water pipes were not insulated water temperature at the tank was different from the toilet assembly, galley assembly and spigot. This effect was most pronounced during the test series TS 3 and TS 7 which were operated at the elevated temperature of 36°C. At elevated operating temperature water temperature decreased by about 10°C from the tank to the toilet assembly, galley assembly and spigot (Table 18).

Table 18: Water temperature at the tank (TIR 1), toilet assembly (TIR 2), faucet (TIR 3), galley assembly (TIR 5) and spigot (TIR 6)

T		Tank (TIR 1)	Toilet Assembly (TIR 2)	Faucet (TIR 3)	Galley Assembly (TIR 5)	Spigot (TIR 6)
[°C]		[°C]	[°C]	[°C]	[°C]	[°C]
20	TS 1	15,7 ± 1,3	18,1 ± 3,2	29,3 ± 2,7	17,5 ± 3,0	18,4 ± 3,6
	TS 4			Aborted		
	TS 5	19,5 ± 1,9	22,9 ± 3,1	31,8 ± 2,9	22,3 ± 2,9	23,5 ± 3,5
	TS 6	17,9 ± 1,4	19,4 ± 1,7	30,1 ± 1,1	19,0 ± 1,6	19,5 ± 1,9
36	TS 2			Aborted		
	TS 3	34,3 ± 0,6	25,1 ± 2,8	33,7 ± 2,0	26,1 ± 2,5	23,0 ± 3,3
	TS 7	33,8 ± 1,8	24,6 ± 2,8	32,9 ± 2,1	26,0 ± 2,6	22,7 ± 3,2

5.2 Reproducibility and microbial characterization of MDWS when operated with unchanged tap water

Figure 4 shows exemplarily a characteristic TCC-course of a drinking water biofilm in comparison to the colony counts HPC, CC 36°C and CC 20°C. The comparison reveals that values of HPC represent constantly about 10 % of the TCC. Values of CC 36°C varied and represent 0,001 to 0,1 % of the TCC. The increase and subsequent decrease of CC 36°C was a typical phenomenon in the present study and may be explained with loss of culturability - bacteria enter the viable but non culturable (VBNC) state (Oliver 2005). Acquisition of CC 20°C generated useless values in general below the detection limit of 1 cfu/cm^2.

This demonstrates that in general the TCC and HPC yield the most reliable data for the assessment of cell and colony counts of a drinking water biofilm.

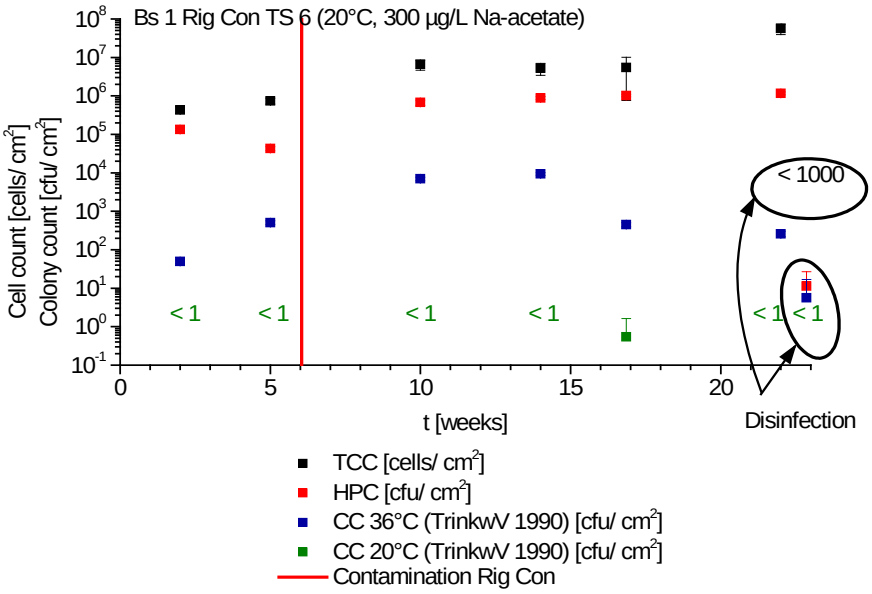

Figure 4: Comparison of cell counts (TCC) and colony counts (HPC, CC 36°C and CC 20°C) of biofilm section Bs 1 from Rig Con at an operating temperature of 20°C and a Na-acetate dosage of 300 µg/L (TS 6). Error bars represent 95 % CI.

Similar difference between total cell numbers and cultivable cells in drinking water biofilms had been found in several investigations (Benölken (2010), Manuel et al. (2007), Wingender and Flemming (2004), Boe-Hansen et al. (2002), Schwartz et al. (1998) and Schaule et al. (1993)).

The test series TS 1 and TS 5 of Rig Ref were operated with unchanged tap water.

HPC of tank water taken from Rig Ref was in the range of 10^3 cfu/mL during TS 1 and TS 5 (Figure 5). Since the HPC of tank water was higher compared to fill-up water (Table 11), it was concluded that bacteria multiply in the tank. The high reproducibility of the HPC indicates constant water quality and operating conditions.

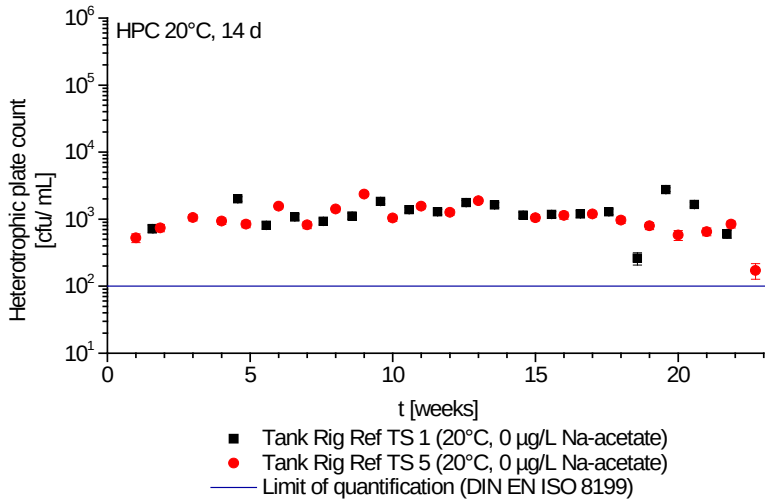

Figure 5: HPC of tank water taken from Rig Ref at 20°C operating temperature without dosage of Na-acetate (TS 1, TS 5). Error bars represent 95 % CI.

Similar to this, Lautenschlager et al. (2010) demonstrated that overnight stagnation of drinking water induced microbial growth and furthermore a change in community composition.

In accordance with the tank water, HPC of biofilms taken from Bs 1 and Bs 2 of Rig Ref had a similar cell density in TS 1 and TS 5 (Figure 6 a, b), whereas the HPC of Bs 2 from TS 5 was slightly lower compared to TS 1 (Figure 6 b). The HPC of Bs 3 was similar in both test series approximately until week 14. From week 14 on the HPC of TS 1 was approximately 1 log-cycle higher than in TS 5 (Figure 6 c). The HPC of Bs 4 showed the highest deviations between TS 1 and TS 5 compared to all other biofilm sections. During TS 1 the HPC remained low until week 5 but increased about 1 log-cycle within 8 weeks and kept stable on that level until end of TS 1. However, the HPC during TS 5 started on a level 1 log-cycle above TS 1. The HPC decreased more than 1 log-cycle until week 10 and stayed constantly low until end of TS 5 (Figure 6 d).

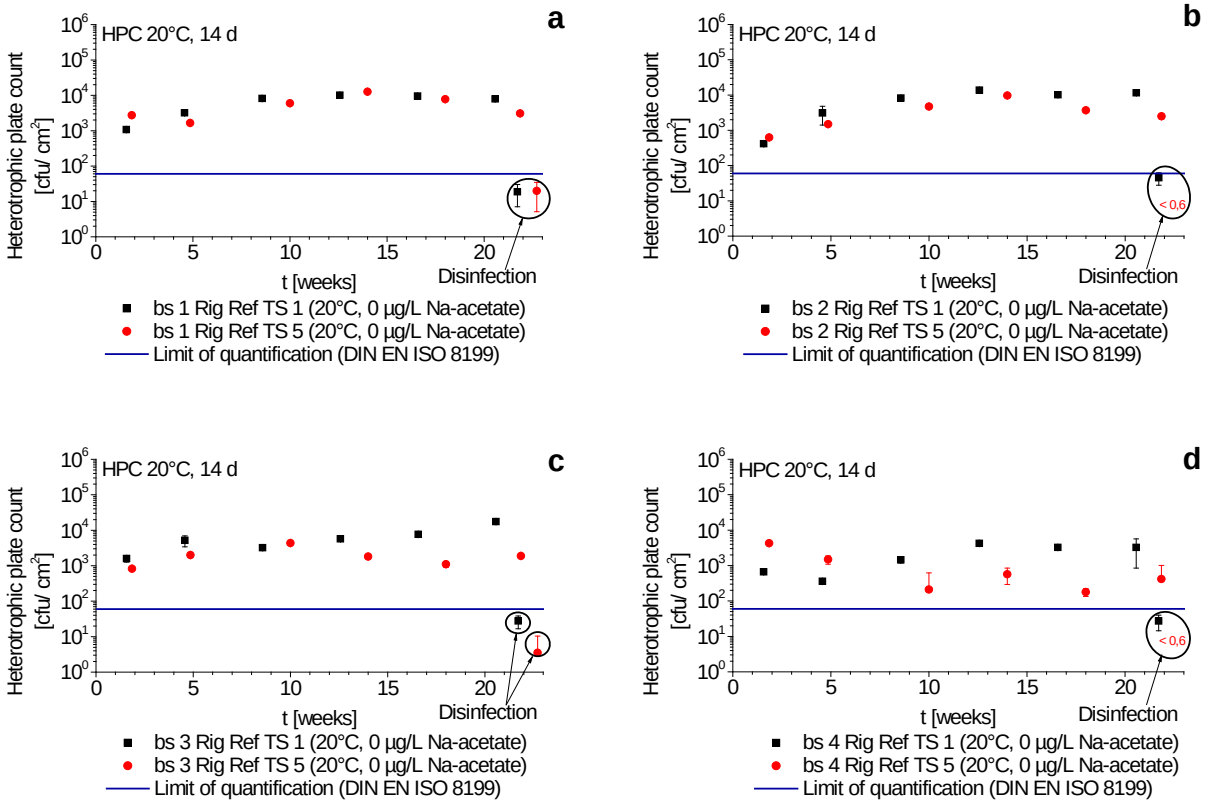

Figure 6: HPC of biofilm sections Bs 1 (a), Bs 2 (b), Bs 3 (c) and Bs 4 (d) from Rig Ref at 20°C operating temperature without dosage of Na-acetate (TS 1, TS 5). Error bars represent 95 % CI.

An explanation for the difference between the test series in Bs 4 might be a microbiological degradation of drawing grease, which is a production residue from pipe manufacturing. I. e. during the first test series the biofilm degraded drawing grease residues from the inner surface of the unused pipes resulting in an increase of cell density. Consequently, during TS 5 no more biodegradable drawing grease residues were present, resulting in a lower HPC of the biofilm especially downstream the CAC-filter.

Figure 7 shows the result of a LC with OCD. The red colored chromatogram shows a characteristic fractioning of DOC from fill-up water consisting of HS at the retention time of 43 min, of BB at 49 min, of LMWC at 53 min and of AC and NC at a retention time of about 60 min onwards. In comparison to the fill-up water, the CAC-filtered water from the spigot (green chromatogram) and ultra pure water used as reference (blue chromatogram) contained only minor amounts of DOC, indicating that the CAC-filter adsorbed almost all DOC from the water.

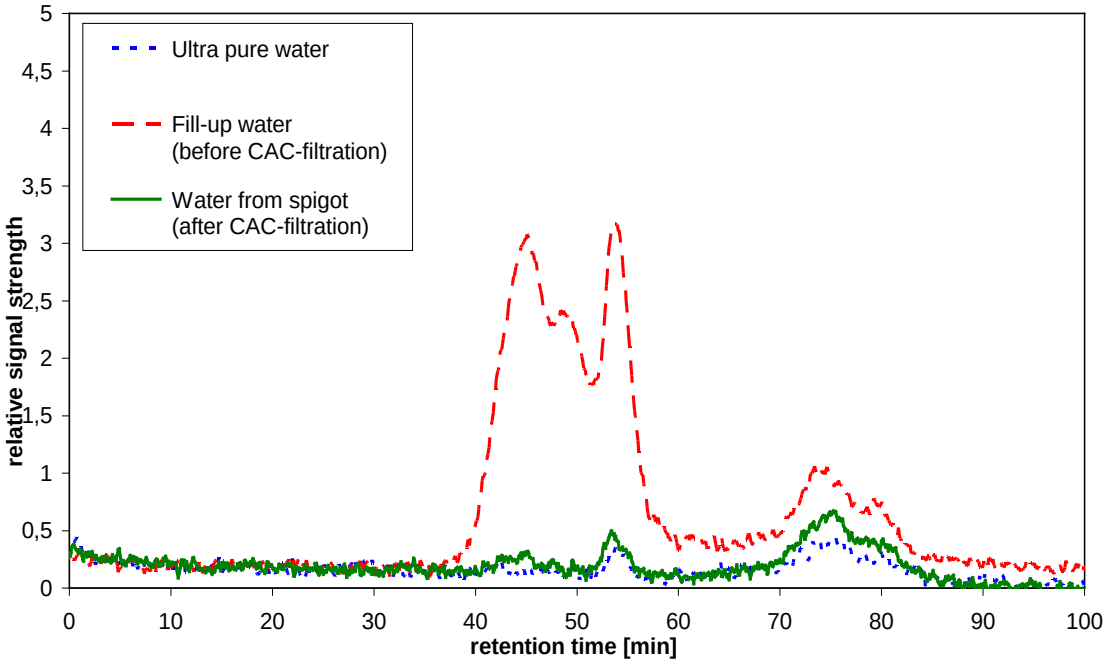

Figure 7: LC-OCD chromatogram of water from MDWS before and after CAC-filtration and ultra pure water (reference)

The absence of biodegradable drawing grease along with an adsorption of DOC by the CAC-filter (Figure 7) led to a decrease of cell density in biofilms downstream the filter over time of operation (Figure 6 d, Bs 4, TS 5). In contrast to Bs 4, there was no difference between TS 1 and TS 5 in Bs 1 (Figure 6 a), meaning that the drawing grease had no impact on the HPC in this biofilm section. Since the HPC of Bs 1 showed no difference between the test series TS 1 and TS 5, the nutrient supply by the unfiltered water seems to be more sufficient than by the drawing grease. The HPC of Bs 2 and Bs 3 represented intermediate states. The impact of drawing grease on HPC was observed earlier and more intense in Bs 3 compared to Bs 2 (Figure 6 b and c). Since Bs 2 had more than twice the amount of flow through compared to Bs 3 (Figure 1 and Table 6) the biofilm in Bs 2 received more than twice the amount of nutrients from bulk water, which might have supported the biofilm formation stronger than the drawing grease.

If DOC was removed by a CAC-filter (adsorbable DOC), biofilm formation was reduced. It was concluded that this DOC fraction supports biofilm formation.

If the water had a very low concentration of DOC, the increase of HPC based on consumption of drawing grease was observed.

In contrast to the HPC (Figure 6), all biofilms had a higher CC 36°C during TS 1 compared to TS 5, supporting the hypothesis of microbiological degradation of drawing grease residues during the first test series (Figure 8).

Since the HPC showed only slight differences between TS 1 and TS 5 it was concluded that the CC 36°C is a more sensitive indicator for identifying microbiological degradation of drawing grease residues.

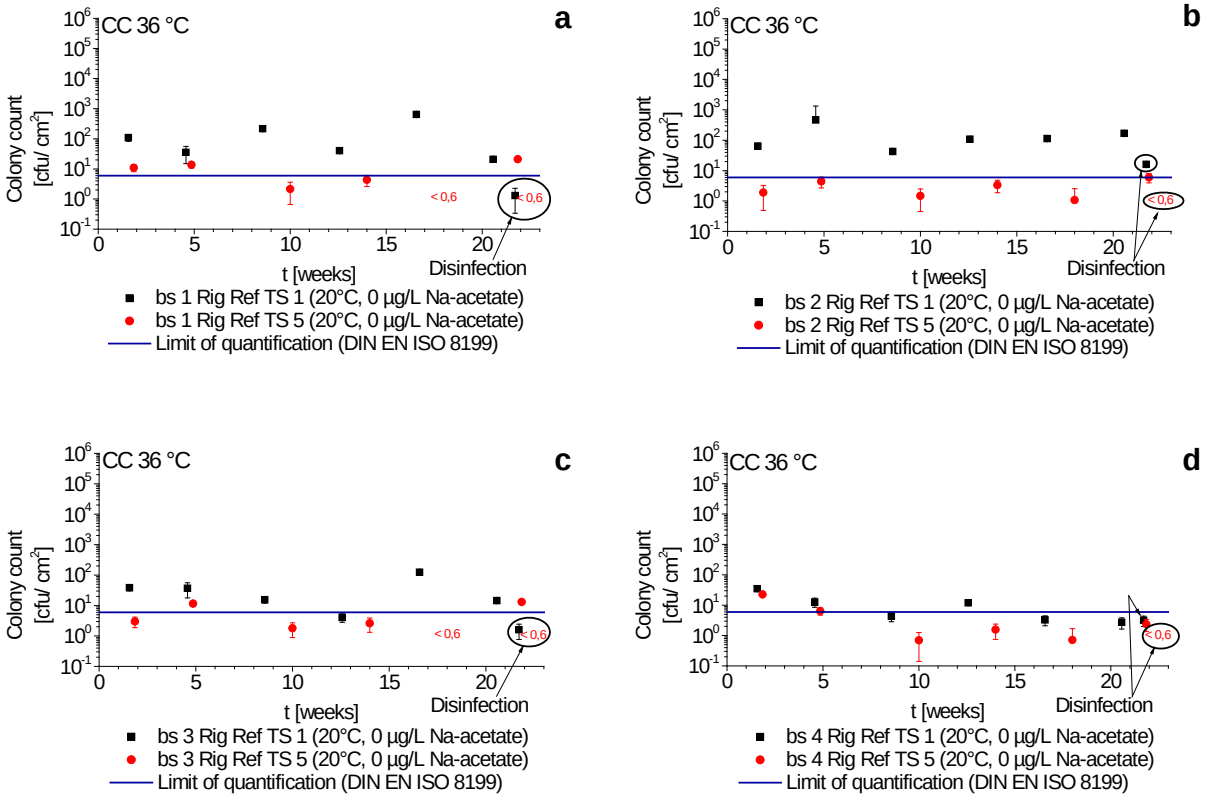

Figure 8: CC 36°C of biofilm sections Bs 1 (a), Bs 2 (b), Bs 3 (c) and Bs 4 (d) from Rig Ref at 20°C operating temperature without dosage of Na-acetate (TS 1, TS 5). Error bars represent 95 % CI.

In general the biofilm densities decreased from Bs 1 to Bs 4 independent of the boarder conditions, which is shown exemplarily for TS 6 from Rig Con (Figure 9). Bs 1 was the pipe with the highest volumetric flow rate (115 L/d), followed by Bs 2 (80 L/d) and subsequently by Bs 3 and Bs 4 (both 35 L/d). Since biofilm cell density and volumetric flow rate decreased from Bs 1 to Bs 3, it was concluded that cell density of the biofilm depended on nutrient supply through the bulk water (Figure 7).

Since Bs 3 and Bs 4 had the same flow rate and Bs 4 had a significant lower biofilm cell density, it was concluded that the DOC adsorption and biological degradation by the CAC-filter (Figure 7) limited biofilm formation downstream the filter.

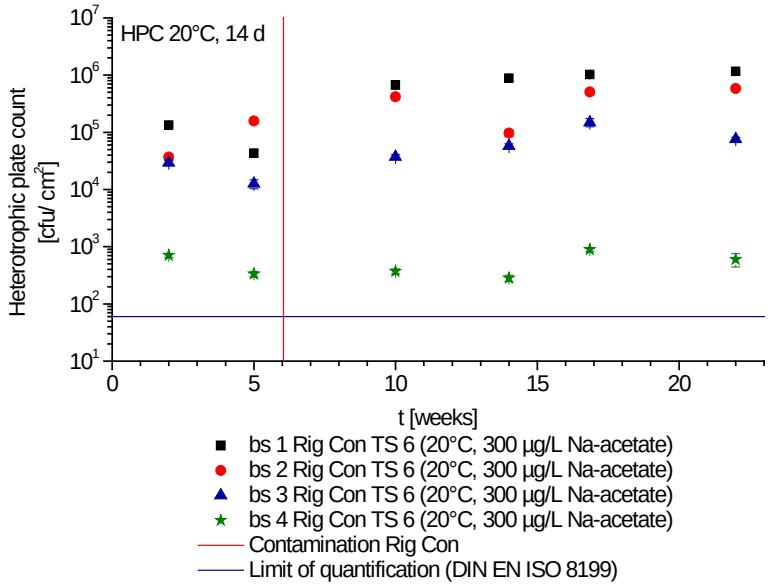

Figure 9: HPC of biofilm sections Bs 1, Bs 2, Bs 3, and Bs 4 from Rig Con at 20°C operating temperature and with a dosage of 300 µg/L Na-acetate (TS 6). Error bars represent 95 % CI.

Similar to this, Lethola et al. (2006) and Manuel et al. (2007) found that higher flow rates lead to better nutrient supply and thus a higher biofilm cell densities.

5.3 Impact of artificial contamination on colony counts

At any of the boundary conditions (Table 7) no impact of the artificial contamination on the parameters CC 20°C, CC 36°C and HPC of any biofilm sample and water samples from tank and spigot had been observed. Additionally, the artificial contamination had no impact on the TCC of biofilm samples (data not shown). The effect of the artificial contamination on the system will be discussed in chapter 5.4 and 5.5.

5.4 Impact of acetate dosage

5.4.1 Total cell counts and colony counts

5.4.1.1 Water

A dosage of 100 µg/L Na-acetate at an operating temperature of 20°C had no impact on the CC 20°C and CC 36°C of the tank water (data not shown). In contrast to that, the HPC of tank water was up to 2 and generally about 1 log-cycle higher with 100 µg/L Na-acetate than without Na-acetate (Figure 10).

It was concluded that Na-acetate, as intended, supported multiplication of planktonic bacteria.

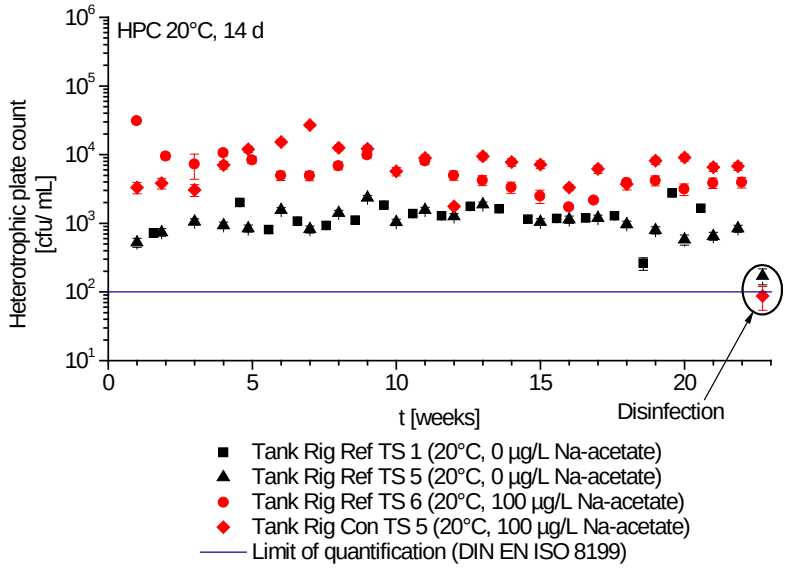

Figure 10: HPC of the tank water from Rig Ref (TS 1, TS 5, TS 6) and Rig Con (TS 5) with and without dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

Also a dosage of 300 µg/L Na-acetate at an operating temperature of 20°C had no impact on the CC 20°C and CC 36°C of water taken from tank, spigot and faucet (data not shown). Furthermore, no impact on the HPC was seen in water taken from spigot (Figure 11 c) which was about 1 log-cycle below the HPC of water taken from tank (Figure 11 a) and faucet (Figure 11 b).

In contrast, with a dosage of 300 µg/L Na-acetate the HPC of water from the tank and faucet were of about the same height as the HPC with a dosage of 100 µg/L Na-acetate until operation week 11. From week 11 on the HPC of the water with 300 µg/L Na-acetate was almost 1 log-cycle higher than with 100 µg/L Na-acetate (Figure 11 a and b).

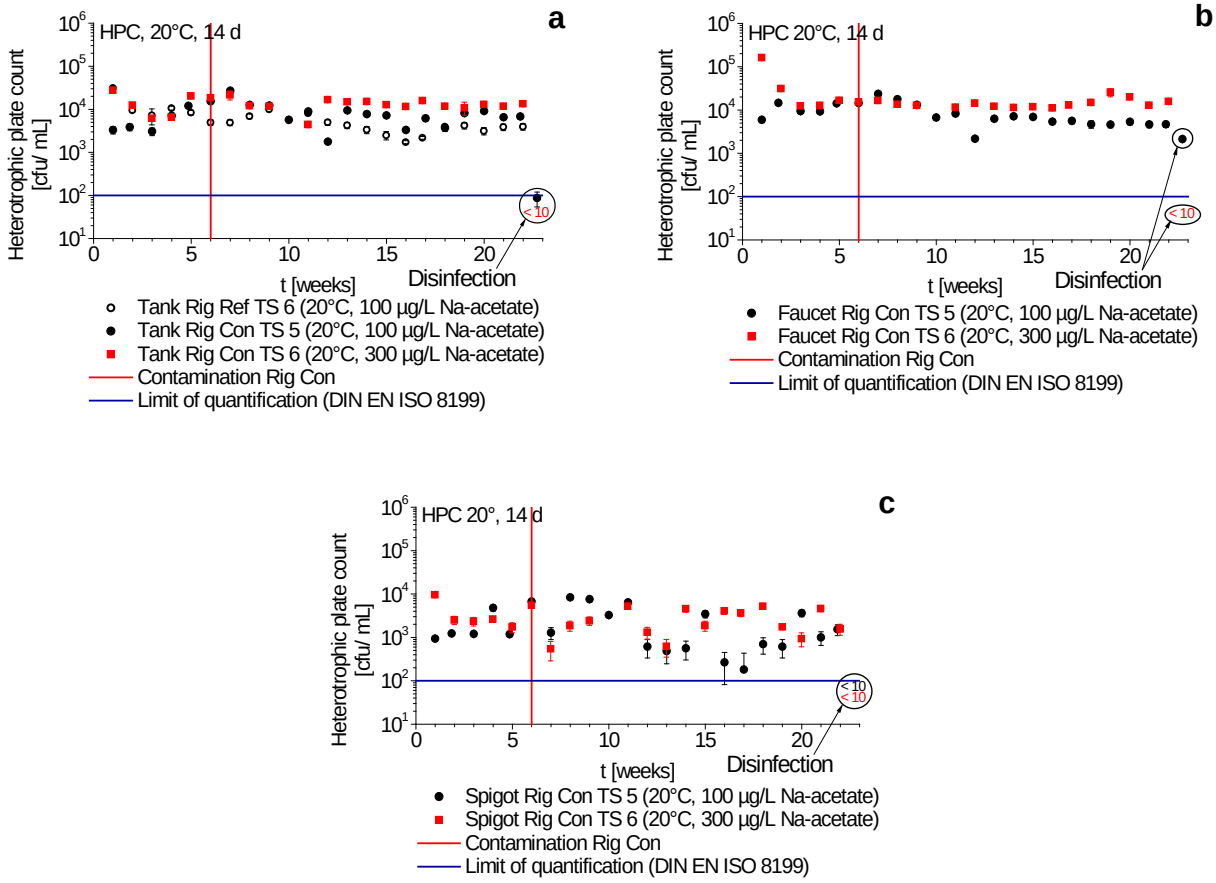

Figure 11: Comparison of HPC with a dosage of 100 µg/L and 300 µg/L Na-acetate and at an operating temperature of 20°C in water taken from the tank (a), faucet (b) and spigot (c). Error bars indicate 95 % CI.

Since the number of planktonic bacteria in the tank and at the faucet increased with increasing acetate dosage but remained low at the spigot over the entire test series, the CAC-filter acted as a barrier for bacteria.

5.4.1.2 Biofilm

In accordance with the tank water, dosage of 100 µg/L Na-acetate at 20°C operating temperature had no impact on CC 20°C and CC 36°C of the biofilms in Bs 1, Bs 2, Bs 3 and Bs 4 (data not shown).

Dosage of 100 µg/L Na-acetate increased the HPC of Bs 1 at least 1 log-cycle over an entire test series of 22 weeks (Figure 12 a). The HPC in Bs 2 was continuously higher with acetate dosage, but was less pronounced between the 10th and 18th week of operation (Figure 12 b).

The acetate dosage increased the HPC of Bs 3 up to 1 log-cycle until the 5th week of operation, afterwards the increase was always less than 1 log-cycle (Figure 12 c). As intended and similar to planktonic bacteria (chapter 5.4.1.1), Na-acetate supported the multiplication of biofilm bacteria.

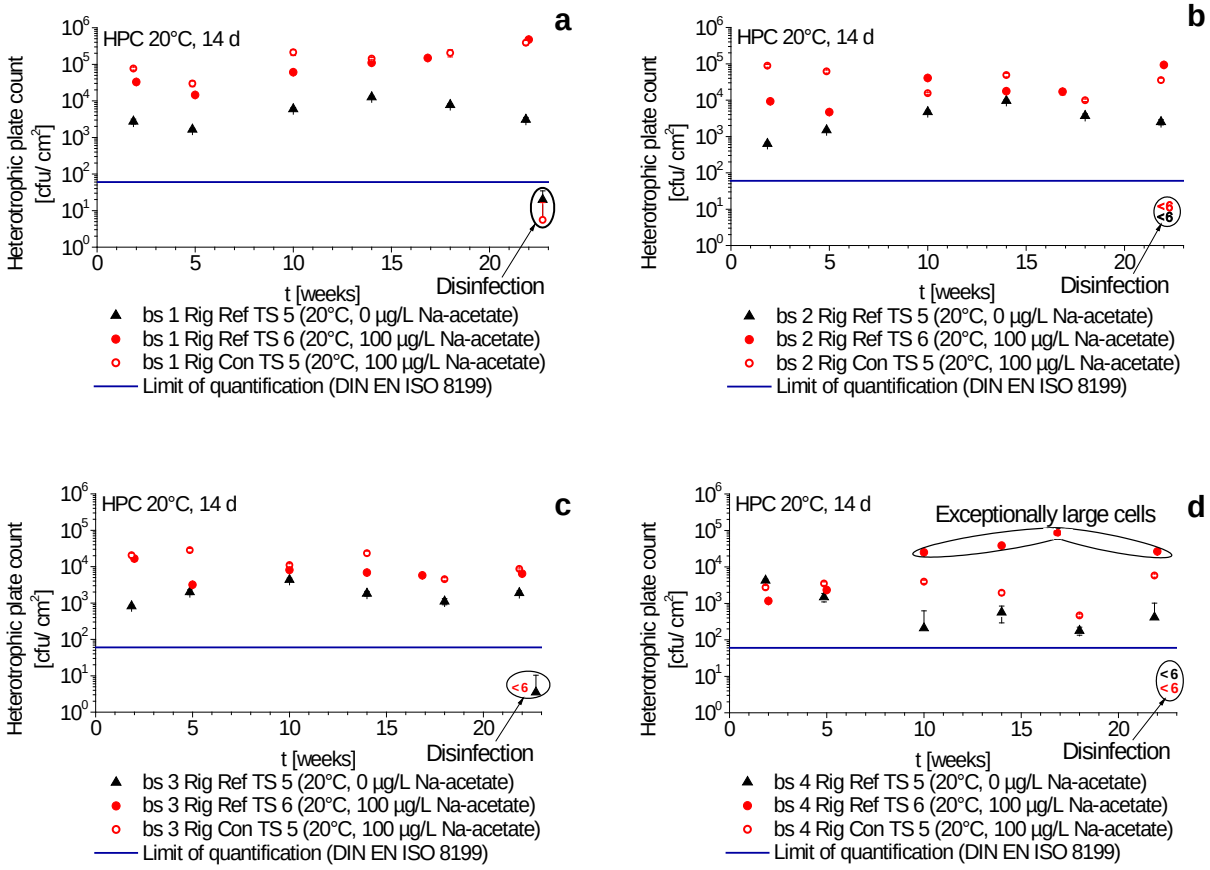

Figure 12: HPC of biofilm sections Bs 1 (a), Bs 2 (b), Bs 3 (c) and Bs 4 (d) from Rig Ref (TS 5, TS 6) and Rig Con (TS 5) with and without dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI

Since the HPC in Bs 3 was not as high as in Bs 2 and since both biofilm sections had the same distance to the acetate dosage (Figure 1 and Table 3), it was concluded that the higher flow through of Bs 2 led to a better nutrient supply of its biofilm.

Explanation of results obtained for Bs 4 is difficult. First of all during TS 6 in Rig Ref four water samples (week 10, 14, 17 and 22) had an unexpected high HPC (Figure 12 d). The bacteria causing the high HPC had been of exceptional large size, they were even larger than bacteria from the well nutrient supplied Bs 1 (Figure 13).

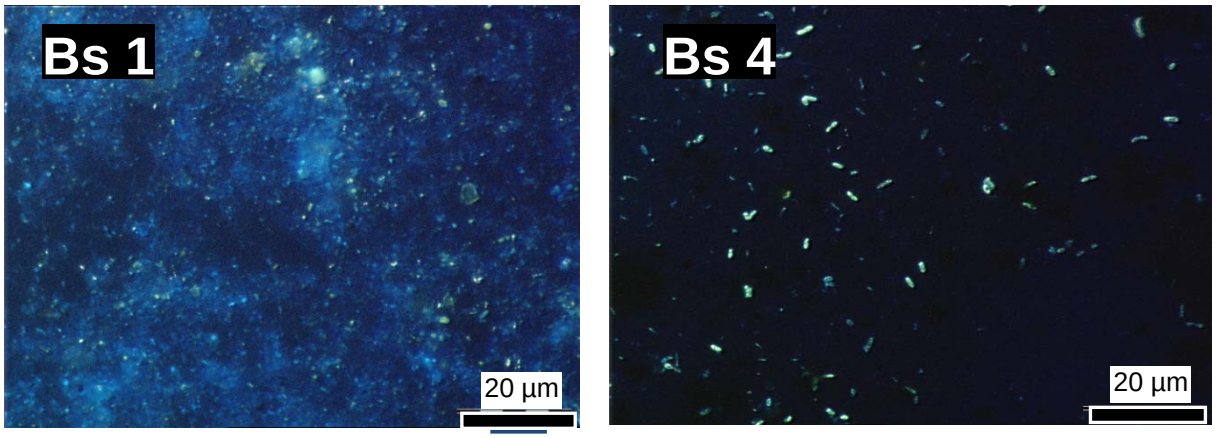

Figure 13: Microscopic pictures of biofilms taken from Bs 1 and Bs 4 of Rig Ref after DAPI staining at operation week 14 of TS 6

If this observation would have been due to a microbiological contamination between the 5th and 10th week of operation, there is only little space for explanation why the colony counts of bacteria remained on this high level and why those bacteria were that large until the 22nd week of operation. A high number of well-developed bacteria would require either a single microbiological contamination combined with a continuous supply with nutrients or a continuous microbiological contamination with already well-developed bacteria. This could be explained by a breakthrough of the CAC-filter. Either the CAC-filter reached its adsorption capacity for organic carbon (OC) resulting in a water, which still contained nutrients for microbial development downstream the filter or the CAC-filter released well developed bacteria. However, a breakthrough is unlikely, since the CAC-filter was a barrier for bacteria - especially large bacteria - and additionally an adsorber for organic OC during all other test series.

Except for the high HPC due to the exceptional large bacteria all other HPC values in Bs 4 were lower compared to the values of Bs 3 in the corresponding test series (Figure 12 d).

After 5 to 10 weeks of operation without acetate dosage at 20°C the HPC decreased in Bs 4 and remained low from week 10 on. Whereas with acetate dosage the HPC decreased from the 10th until the 18th week of operation to the same level as without acetate dosage but increased right away until week 22 to the starting value (Figure 12 d).

In conclusion, although an acetate dosage of 100 µg/L at 20°C operating temperature increased the HPC of biofilms downstream the CAC-filter, the filter reduced the biofilm

formation over the entire test series of 22 weeks corresponding to a filtered water volume of 5400 L.

5.4.2 Facultative pathogens

5.4.2.1 Water

Water from tank and spigot

At an operating temperature of 20°C none of the contaminants were detected in the tank water, neither with a dosage of 100 µg/L nor with 300 µg/L Na-acetate (data not shown).

As observed for the tank water, none of the contaminants were detected in water from the spigot (data not shown).

Water from faucet

Even though the HPC at the faucet increased with increasing acetate dosage, the concentrations of the artificially applied contaminants *P. aeruginosa* and *E. faecalis* were lower in water with 300 µg/L Na-acetate. Additionally the contaminants were detected in fewer samples (Figure 14 a and b).

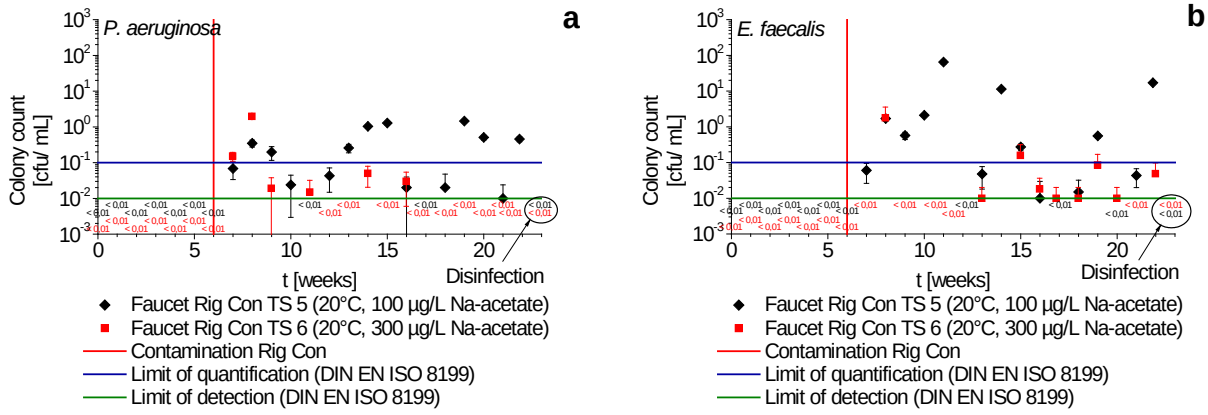

Figure 14: Colony counts of *P. aeruginosa* (a) and *E. faecalis* (b) in water taken from the faucet of Rig Con with dosages of 100 µg/L and 300 µg/L Na-acetate at an operating temperature of 20°C. Error bars indicate 95 % CI.

P. aeruginosa and *E. faecalis* had the ability to persist at the faucet. This was concluded from sampling, which had always been done seven days after every artificial contamination (chapter 4.5). During this time 350 L of water flowed through the faucet (Table 6). A literature review conducted in 2009 by Mena and Gerba pointed out that detection of *P. aeruginosa* in drinking water is common even in bottled water. A recently published nation wide survey revealed that 2,9 % of 3468 water samples from public buildings in Germany contained pseudomonades. In contrast to pseudomonades, enterococci were detected in only 0,3 % (n=1177) (Völker et al. 2010). The ability of

P. aeruginosa to grow in drinking water was already demonstrated in 1976 by Botzenhart and Kufferath and was later confirmed by van der Kooij et al. (1982 b) and Warburton et al. (1994). Additionally, growth of *P. aeruginosa* was shown in bottled water (Warburton et al. (1994), Moreira et al. (1994) and Tamagnini and Gonzáles (1997)) and even in distilled water (Favero et al. (1971)).

The pathogens *P. aeruginosa* and *E. faecalis* preferred the condition at a lower acetate dosage. Accordingly, Edberg et al. (1996) postulated that *P. aeruginosa* favors an environment of low ionic strength and low concentrations of sugars and proteins. Van der Kooij (1982 b) stated that growth of *P. aeruginosa* depends on concentration and quality of organic carbon. In contrast to what might be expected, the concentration of *P. aeruginosa* that will develop by digestion of a mixture of organic carbon is not linearly correlated to the concentration of the carbon source.

Beside low carbon concentrations, a lower number of total bacteria in biofilm (chapter 5.4.1.2) and water phase (chapter 5.4.1.1) and thus fewer competitors for the substrate might be a reason for higher numbers of *P. aeruginosa* and *E. faecalis*. Van der Kooij et al. (1982 b) showed that the autochthonous planktonic flora or even *P. fluorescens* strain P17 inhibit *P. aeruginosa* in attaining large numbers in drinking water. Additionally, high concentrations of *P. aeruginosa* will decline in the presence of the autochthonous flora (de Vicente et al. 1988). Moreira et al. (1994) gained varying results for *P. aeruginosa*. They found *P. aeruginosa* lost 99 % of its culturability in the presence of autochthonous bacteria in a mineral water in polyvinyl chloride (PVC) bottles, while *P. aeruginosa* remained culturable in the absence of autochthonous bacteria. In contrast to the PVC bottles, *P. aeruginosa* lost 99,9 % of its culturability in the same mineral water in the absence of autochthonous bacteria in glass bottles. Additionally, Moreira et al. (1994) showed that *P. aeruginosa* multiplied when suspended in sterile tap water and stored in the same glass bottles in which *P. aeruginosa* lost culturability when suspended in the mineral water. Furthermore, culturability of *P. aeruginosa* depends on plumbing material (Moritz et al. (2010) and Benölken (2010)) and on the concentration of copper ions in the water phase (Dwidjosiswojo et al. (2011)).

This clearly demonstrates that beside autochthonous bacteria, water composition and material in contact with water influence the culturability of *P. aeruginosa*.

The contaminant *E. coli* was never detected (data not shown). According to this finding, Baumgartner and Grand (2006) and Liguori et al. (2010) sampled water dispensers and found no contamination with the indicator organism *E. coli*, whereas *P. aeruginosa* was detected at several sampling points. In contrast, Lévesque et al. (1994) detected *E. coli* in approximately 30 % of all samples taken from coolers in a survey conducted in Canada.

5.4.2.2 Biofilm

P. aeruginosa was detected once in the biofilms of Bs 1, Bs 2 and Bs 3, respectively, four weeks after first artificial contamination in TS 6 with a dosage of 300 µg/L Na-acetate but never in TS 5 with 100 µg/L (Table 19). The concentration of *P. aeruginosa* at 20°C operating temperature and with a dosage of 300 µg/L Na-acetate was in Bs 1 the highest followed by Bs 2 and subsequently by Bs 3 which was in accordance with the decreasing cell densities of the biofilm (chapter 5.2, Figure 4).

In TS 5 with a dosage of 100 µg/L Na-acetate, *E. faecalis* was detected once in Bs 1 four weeks after first artificial contamination and twice in Bs 2 after four and eight weeks after first artificial contamination. With a dosage of 300 µg/L Na-acetate, *E. faecalis* was detected twice in Bs 2 after 11 and 16 weeks after first artificial contamination. Any time *E. faecalis* has been detected the concentration was 0,06 cfu/cm^2 which is equal to the limit of detection (Table 19).

E. coli had never been detected in any of the biofilm sections (Table 19).

None of the contaminants were detected in Bs 4 (Table 19), neither with a dosage of 100 µg/L nor with 300 µg/L Na-acetate, indicating that the CAC-filter acted as a barrier for the contaminants.

Table 19: Colony counts of contaminants in the biofilm sections of Rig Con at 20°C operating temperature with a dosage of 100 µg/L and 300 µg/L Na-acetate, respectively. Numbers in brackets represent the week of detection after first artificial contamination.

	20°C, 100 µg/L Na-acetate (TS 5)				**20°C, 300 µg/L Na-acetate (TS 6)**			
	Bs 1	**Bs 2**	**Bs 3**	**Bs 4**	**Bs 1**	**Bs 2**	**Bs 3**	**Bs 4**
	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2
P. aeruginosa	< 0,06	< 0,06	< 0,06	< 0,06	23,25 (4)	0,86 (4)	0,08 (4)	< 0,06
E. faecalis	0,06 (4)	0,06 (4) 0,06 (8)	< 0,06	< 0,06	< 0,06	0,06 (11) 0,06 (16)	< 0,06	< 0,06
E. coli	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06

Analysis with FISH resulted in a detection of *P. aeruginosa* solely in Bs 1 at 20°C operating temperature and with a dosage of 300 µg/L Na-acetate four weeks after first artificial contamination (Figure 15), supporting the results from cultivation method (Table 19). The percentage of *P. aeruginosa* was less than 1 % of the TCC, representing a concentration of $< 6{,}6 \cdot 10^4$ cells/cm^2. The FISH-results leave open how high the cell density up to $6{,}6 \cdot 10^4$ cells/cm^2 actually was. However, it cannot be excluded that the concentration of *P. aeruginosa* in the biofilm section Bs 1, detected with FISH, might have been higher than detected with the cultivation method (23 cfu/cm^2, see Table 19). In contrast to the results from cultivation method (Table 19), *P. aeruginosa* was not detected with FISH in the biofilm sections Bs 2 and Bs 3. This might be due to two reasons. Firstly, the concentration of *P. aeruginosa* was too low. Or with other words, the concentration of *P. aeruginosa* in the biofilm sections Bs 2 and Bs 3 of Rig Con represent a too small proportion of the TCC for detection with FISH. Secondly, *P. aeruginosa* remained undetected due to a too low ribosome content, which is often found in starved cells as, discussed by Glöckner et al. (1999). The latter explanation requires a heterogenic population within the test rig. Since the cell density of the biofilm depended on the nutrient supply through the bulk water (chapter 5.2) and the cell density of Bs 1 was the highest, it might be that the nutrient supply of *P. aeruginosa* in Bs 1 was better compared to Bs 2 and Bs 3, resulting in a higher ribosome content of *P. aeruginosa* in Bs 1. However, Manz et al. (1993) demonstrated that planktonic bacteria and biofilm bacteria from oligotrophic environments like drinking water systems can be detected by FISH, although about 60 % of planktonic cells and about 30 % of biofilm cells remained undetected.

Identical to results from cultivation method (Table 19), *P. aeruginosa* was not detected with FISH at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 5) (data not shown).

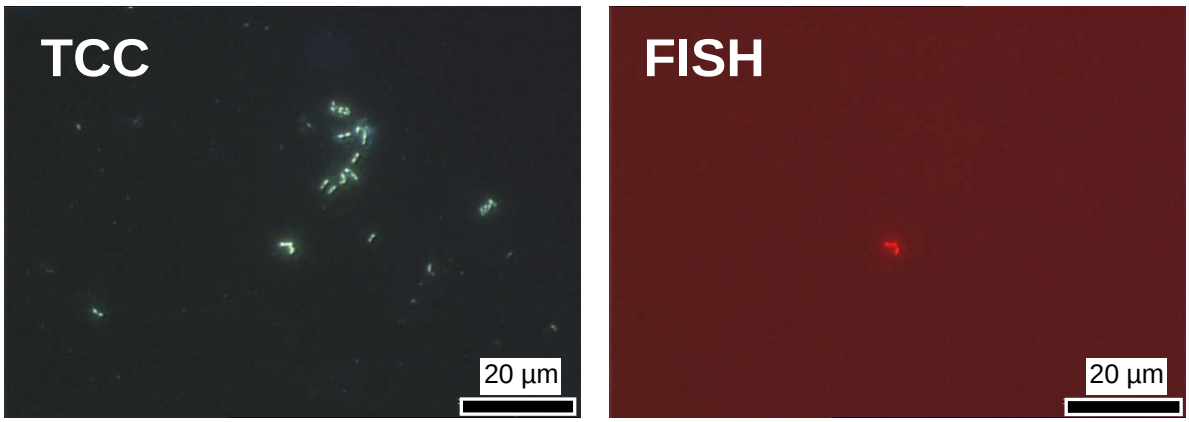

Figure 15: Microscopic pictures of biofilm taken from biofilm section BS 1 of Rig Con at operation week 10, four weeks after first artificial contamination of TS 6 (20°C, 300 µg/L Na-acetate). DAPI stained bacteria (left) and cells hybridized with probe Psae16S-182 (right) from the identical microscopic field.

Results of contaminants at 20°C operating temperature can be summarized as follows:

At 20°C operating temperature a retrograde contamination with *P. aeruginosa* and *E. faecalis* occurred at a dosage of 100 µg/L and 300 µg/L Na-acetate. However, the retrograde contamination was limited to the biofilm.

The cell density of *P. aeruginosa* might have been dependent on the total cell density of the biofilm.

A systemic contamination of the entire system with *P. aeruginosa* and *E. faecalis* including a contamination of the water did not occur. According to this, Hoadley (1977) suggests faucets in hospitals as a favoured habitat of *P. aeruginosa*. Later, several studies confirmed Hoadley's suggestion. *P. aeruginosa* were regularly detected in water taken from faucets and never in corresponding mains (Halabi et al. (2001), Reuter et al. (2002) and Trautmann et al. (2005)). Furthermore Reuter et al. (2002) demonstrated that faucets were contaminated by patients and patients were contaminated by water taken from already contaminated faucets.

Similar to the findings in the environment of intensive care units, Liguori et al. (2010) detected *P. aeruginosa* regularly in water taken from faucets of water dispensers, whereas *P. aeruginosa* was detected only once out of 48 samples of the corresponding water supply lines.

Since no cultivation independent method like FISH had been applied for the detection of *E. faecalis* and *E. coli*, the bacteria might be present in a VBNC state even at high concentrations, as demonstrated for *E. coli* in biofilms by Juhna et al. (2007).

5.5 Impact of temperature

The impact of temperature on the microbiology of both biofilm and water phase was investigated at three different OC-concentrations:

1. At a low OC-concentration without acetate dosage in order to investigate the general effect of an elevated temperature (chapter 5.5.1).
2. At an elevated OC-concentration with a dosage of 100 µg/L Na-acetate in order to investigate the combination of elevated temperature and OC-concentration (chapter 5.5.2).
3. At a high OC-concentration with a dosage of 300 µg/L Na-acetate in order to investigate a case with poor water quality in combination with an elevated temperature level (chapter 5.5.3).

5.5.1 Impact of temperature at low OC-concentration

5.5.1.1 Total cell counts and colony counts

5.5.1.1.1 Water

The increased operating temperature of 36°C without dosage of Na-acetate had no effect on the CC 20°C of the water phase (data not shown).

Simultaneously to the increased operating temperature, the CC 36°C of water taken from the tank increased as well, especially until approximately 12th week of operation. From week 12 on the CC 36°C of the elevated operating temperature varied and decreased subsequently to the level of the CC 36°C at 20°C operating temperature. The limiting value of 100 cfu/mL (TrinkwV 2001) was exceeded in only four cases during the test series (Figure 16 a).

The HPC of tank water showed similar results as the CC 36°C. At the elevated operating temperature of 36°C the HPC was up to 1 log-cycle higher than at 20°C until week 13. From week 13 on the HPC at the elevated temperature decreased to a similar level as for the low operating temperature (Figure 16 b).

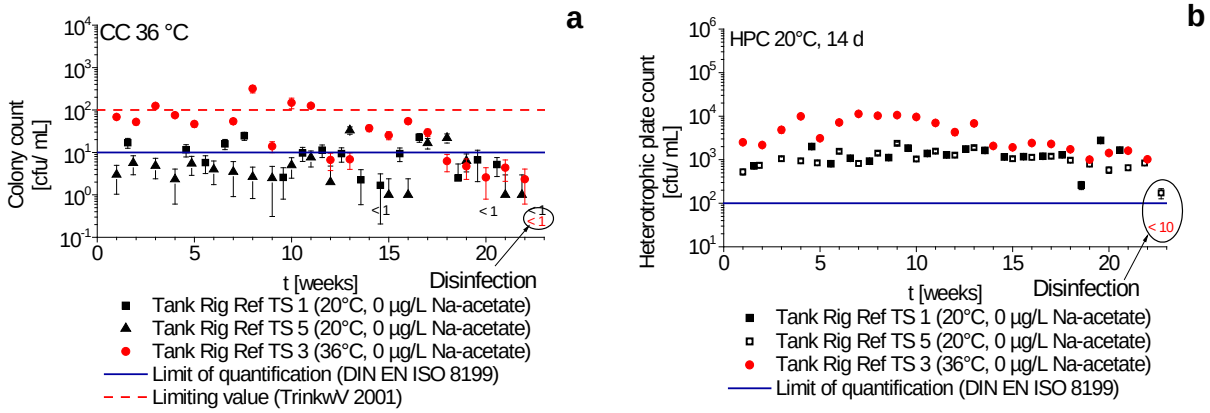

Figure 16: CC 36°C (a) and HPC (b) of tank water from Rig Ref at low (TS 1, TS 5) and elevated operating temperature (TS 3) without acetate dosage. Error bar represent 95 % CI.

Studies conducted by Reasoner et al. (1989) and Power and Nagy (1999) found positive correlation for water temperature and concentration of planktonic heterotrophic bacteria in municipal drinking water distribution systems.

Carter et al. (2000) confirmed a positive correlation for water temperature and concentration of planktonic heterotrophic bacteria cultured on “R2A”-Medium according to Reasoner and Geldreich (1985). In contrast to the “R2A”-Medium, cultivation of heterotrophic bacteria on plate count agar (PCA) and on sheep blood agar (TSA-SB) did not correlate with water temperature.

Even though the CC 36°C and HPC were higher at elevated operation temperature they decreased with operation time subsequently to the level which was observed for 20°C operation temperature (Figure 16 a and b). This effect was not described by above cited studies (Reasoner et al. (1989), Power and Nagy (1999) and Carter et al. (2000)).

An explanation for the decrease of colony counts (CC 36°C and HPC) with operation time is probably not a decrease in concentration of viable bacteria, it is rather more a loss of culturability as shown by Byrd et al. (1991).

The loss of culturability could not be validated in this study because no cultivation independent method like TCC had been applied for water samples.

5.5.1.1.2 Biofilm

Compared to the lower operating temperature of 20°C, the elevated temperature of 36°C without acetate dosage had only little influence on the parameters measured for the biofilm. Only a tendency towards increased total cell counts was observed (data not shown).

5.5.1.2 Facultative pathogens

Rig Con was never operated without acetate dosage (chapter 4.3). Consequently, there is no data basis to assess the behavior of the artificial contamination under this boundary condition.

5.5.2 Impact of temperature at elevated OC-concentration

5.5.2.1 Total cell counts and colony counts

5.5.2.1.1 Water

Tank water

The combination of a dosage of 100 µg/L Na-acetate and an operating temperature of 36°C had neither an effect on the CC 20°C of the water phase nor on the CC 20°C of the biofilm (data not shown).

Simultaneously to the elevated operating temperature at a dosage of 100 µg/L Na-acetate, the CC 36°C of water taken from tank was over the entire test series of 22 weeks 1 to 2 log-cycle higher compared to the lower operating temperature. The limiting value of 100 cfu/mL (TrinkwV 2001) was exceeded regularly during the test series at elevated operating temperature (Figure 17 a). The elevated CC 36°C at elevated temperature with acetate dosage (Figure 17 a) was consistent over 22 weeks and more pronounced than without acetate dosage (Figure 16 a).

The HPC revealed inconsistent results. At an operating temperature of 20°C tank water from Rig Ref and Rig Con had the same HPC. However, at 36°C operating temperature the HPC of tank water from Rig Ref was more or less the same whereas it was about 1 log-cycle higher in Rig Con (Figure 17 b). The HPC at 36°C in Rig Ref was in the range of the HPC of both test rigs at 20°C. The HPC of the tank water remained independent of the artificial contamination since no decrease or increase occurred after start of weekly contamination at the faucet of Rig Con at operation week 6.

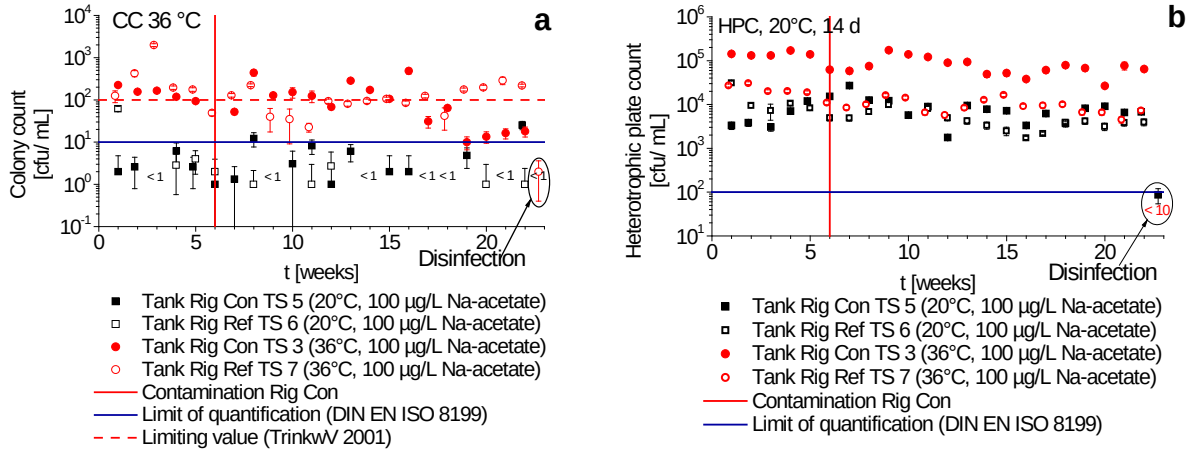

Figure 17: CC 36°C (a) and HPC (b) of tank water from Rig Ref (TS 6, TS 7) and Rig Con (TS 3, TS 5) at low (TS 5, TS 6) and elevated (TS 3, TS 7) operating temperature always with dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

In contrast to the loss of culturability of planktonic bacteria at elevated operating temperature, as mentioned in chapter 5.5.1.1.1, an elevated OC-concentration seemed to sustain culturability. Gibbs et al. (1993) investigated a municipal drinking water system in the UK. They found out that the AOC concentration did not correlate with culturability, rather a recovery from chlorination seemed to be the reason for an increase in culturability in that municipal drinking water system. Boualam et al. demonstrated in 2001 that coliform bacteria loose culturability in low nutritive waters. Additionally they found that the higher the initial DOC concentration was, the slower was the loss in culturability. Ellis et al. (2000) tested three of the commonly accepted primary carbon sources in aquatic environments (amino acids, carbohydrates and humic substances) and found an increased culturability of planktonic heterotrophic bacteria due to each carbon source.

However, the loss or sustentation of culturability could not be validated because no cultivation independent method like TCC had been applied for water samples.

Water from spigot

At elevated operating temperature and a dosage of 100 µg/L Na-acetate the CC 36°C of water taken from the spigot was higher compared to the CC 36°C at the operating temperature of 20°C. For the vast majority of samples the CC 36°C remained below the limiting value of 100 cfu/mL (TrinkwV 2001) (Figure 18 a).

The HPC of the spigot showed no consistent temperature dependency (Figure 18 b).

Although the tank water was highly loaded with bacteria at the elevated operating temperature (Figure 17 b), the concentration in water taken from the spigot remained low indicating that the CAC-filter still acted as a barrier over the entire test series of 22 weeks. The CAC-filter reduced the HPC at elevated operating temperature by about 2 log-cycles or 99 % (Figure 17 b, Figure 18 b).

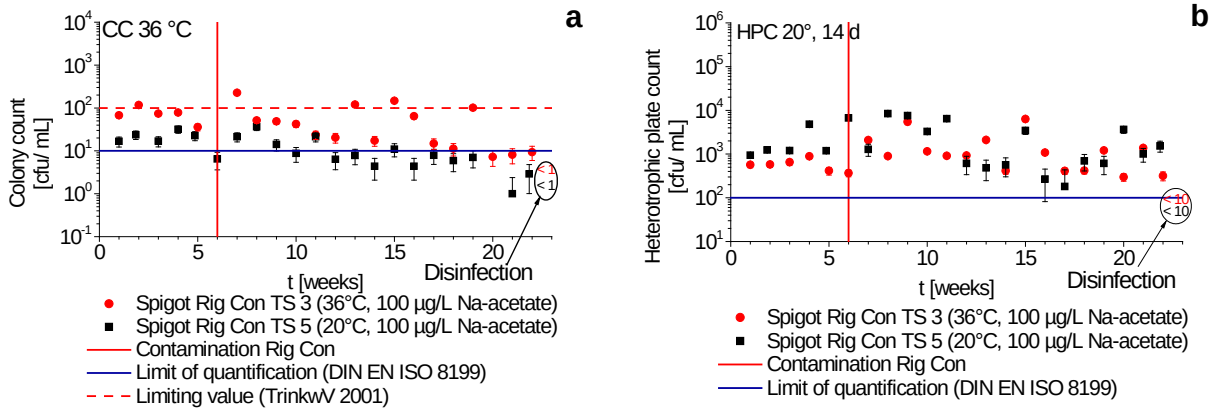

Figure 18: CC 36°C (a) and HPC (b) of water taken from the spigot of Rig Con at low (TS 5) and elevated (TS 3) operating temperature always at a dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

Water from faucet

Comparable to the tank water, the CC 36°C of water with 100 µg/L Na-acetate taken from the faucet was in general 1 to 2 log-cycles higher at the elevated operating temperature. The CC 36°C increased so pronounced that the limiting value of 100 cfu/mL (TrinkwV 2001) was usually exceeded (Figure 19 a). Similar to the tank water (Figure 17 a) an elevated OC-concentration at elevated operating temperature seemed to sustain culturability.

In accordance with the tank water from TS 3 for Rig Con (Figure 17 b) the HPC at the faucet was about 1 log-cycle higher over the entire test series of 22 weeks due to the increased operating temperature of 36°C (Figure 19 b).

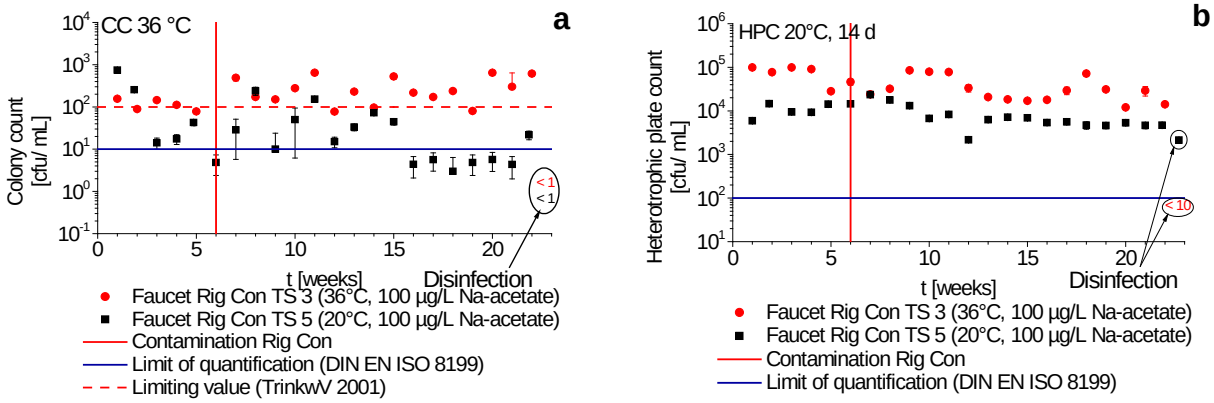

Figure 19: CC 36°C (a) and HPC (b) of water taken from the faucet of Rig Con at low (TS 5) and elevated (TS 3) operating temperature with a dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

5.5.2.1.2 Biofilm

The elevated operating temperature of 36°C and a dosage of 100 µg/L Na-acetate had no impact on the CC 20°C, CC 36°C, HPC and TCC of the biofilm section Bs 2, Bs 3 and Bs 4 (data not shown).

In Bs 1 the CC 20°C, HPC and TCC remained unchanged (data not shown). The CC 36°C of Bs 1 was up to 1 log-cycle higher at the elevated operating temperature until operation week 10. Afterwards it decreased to the level of the colony counts at 20°C operating temperature (Figure 20).

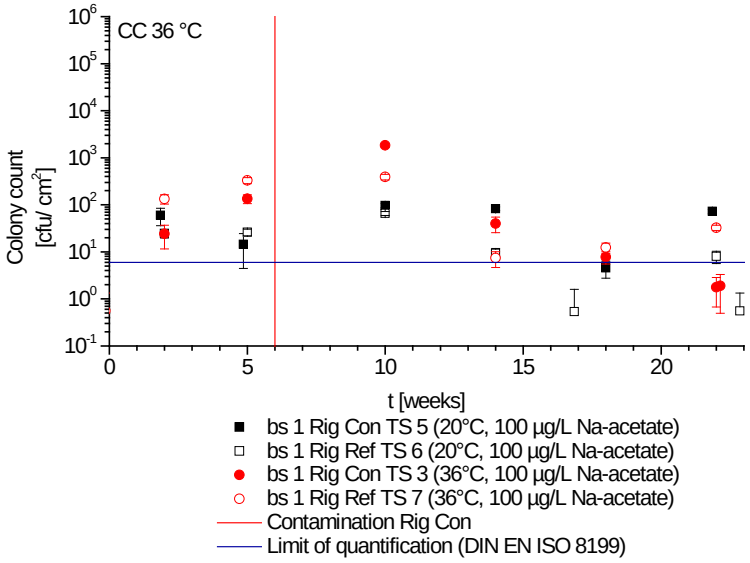

Figure 20: CC 36°C of biofilm from Bs 1 of Rig Ref (TS 6, TS 7) and Rig Con (TS 3, TS 5) at low (TS 5, TS 6) and elevated (TS 3, TS 7) operating temperatures always with a dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

The CC 36°C was a sensitive parameter for tank water samples at the elevated water temperature as shown in chapter 5.5.1 and 5.5.2. Since, the temperature-dependent increase of CC 36°C was solely observed in Bs 1 and the water temperature decreased from 34°C (tank) to 25°C (lavatory assembly) it was concluded that the missing thermal insulation of the biofilm sections led to comparable cell densities in the biofilms of Bs 2, Bs 3, and Bs 4 at room and elevated operating temperature.

Since the CC 36°C increased and subsequently decreased again and the TCC remained constant, bacteria lost culturability from operation week 10 onward. According to this Benölken (2010) found a loss of culturability of biofilm bacteria from different pipe materials.

The loss of culturability at 36°C operation temperature and elevated OC-concentration seems to be contradictive to the results found for water samples (chapter 5.5.2.1.1).

5.5.2.2 Facultative pathogens

5.5.2.2.1 Water

Water from tank and spigot

At a dosage of 100 µg/L Na-acetate and an operating temperature of 20°C, none of the contaminants were detected in water taken from tank and spigot (Table 20).

In contrast to the test series at low operating temperature *P. aeruginosa* was detected in week 7, 8 and 16 after first artificial contamination at elevated operating temperature (Table 20).

Additionally, *E. faecalis* was detected once in water taken from tank in week 9 after first artificial contamination (Table 20).

If a contaminant was detected, its concentration had been at the detection limit of 0,01 cfu/mL or very close to it.

E. coli was never detected in water samples taken from tank and spigot (Table 20).

None of the contaminants were detected at the spigot (Table 20).

Table 20: Concentration of contaminants in water samples taken from tank and spigot of Rig Con with a dosage of 100 µg/L Na-acetate and at an operating temperature of 20°C and 36°C. Numbers in brackets represent the week of detection after first artificial contamination.

	20°C, 100 µg/L Na-acetate (TS 5)		**36°C, 100 µg/L Na-acetate (TS 3)**	
	tank water	**spigot**	**tank water**	**spigot**
	cfu/mL	cfu/mL	cfu/mL	cfu/mL
P. aeruginosa	< 0,01	< 0,01	0,01 (7) 0,03 (8) 0,01 (16)	< 0,01
E. faecalis	< 0,01	< 0,01	0,01 (9)	< 0,01
E. coli	< 0,01	< 0,01	< 0,01	< 0,01

Although a retrograde contamination of the tank water occurred at elevated operating temperature the concentration of *P. aeruginosa* and *E. faecalis* did not increase over operation time, indicating that they did not proliferate in the tank water. This might be due to competition for substrate with autochthonous bacteria as discussed in chapter 5.4.2.1.

Simultaneous to the retrograde contamination of the tank water at elevated operating temperature and a Na-acetate dosage of 100 µg/L the concentration of *P. aeruginosa* at the faucet increased by 2 to 3 log-cycles (Figure 21 a). As already discussed in chapter 5.4.2 the faucet seemed to be a favored habitat of *P. aeruginosa* and *E. faecalis*.

Because none of the contaminants were detected in water taken from the spigot the CAC-filter acted as a barrier.

Water from faucet

With a dosage of 100 µg/L Na-acetate and at an operating temperature of 36°C the concentration of *P. aeruginosa* increased by 2 to 3 log-cycles compared to the operating temperature of 20°C (Figure 21 a). The higher concentration demonstrates that *P. aeruginosa* did not only retain at the faucet, it rather proliferated although the concentration of other competing bacteria (CC 36°C, HPC) was higher as well. In contrast to this finding, Edberg et al. (1996) stated that *P. aeruginosa* favors low water temperatures. A nation wide survey conducted by Völker et al. (2010) revealed no difference between the percentage of detected *P. aeruginosa* from cold water and warm water, taken from in-building distribution systems of public buildings in Germany.

In contrast to *P. aeruginosa,* the concentration of *E. faecalis* was not remarkably influenced by the elevated operating temperature. In comparison to 20°C operating temperature *E. faecalis* was detected 3 weeks later at the elevated operating temperature in the same range of concentration (Figure 21 b). In contrast to this, the survey conducted by Völker et al. (2010) showed that enterococci were never detected from warm water taken from in-building distribution systems. It has to be mentioned that 52,1 % of the sampled warm water systems had a water temperature above 50°C.

The contaminant *E. coli* was never detected in water samples from the faucet.

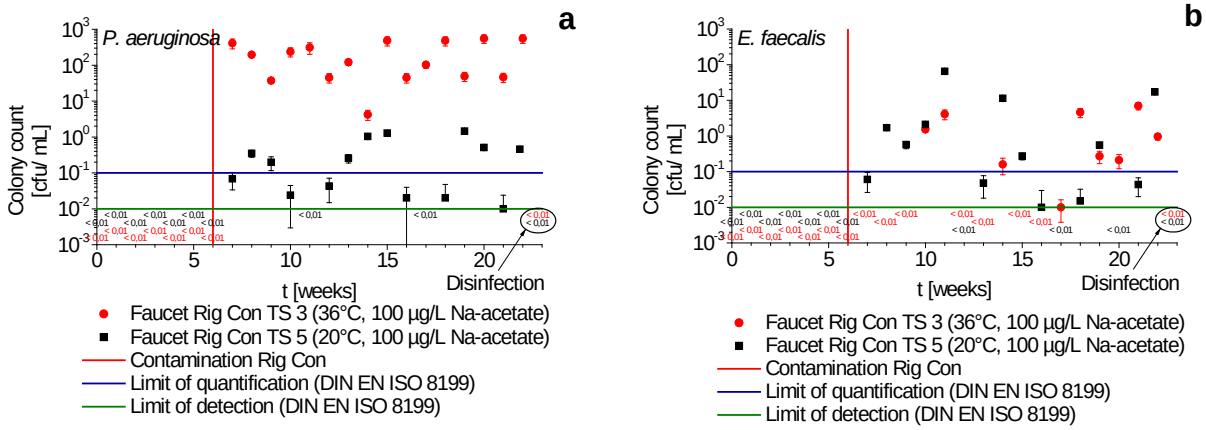

Figure 21: Colony counts of *P. aeruginosa* (a) and *E. faecalis* (b) of water taken from the faucet of Rig Con at low (TS 5) and elevated (TS 3) operating temperature with a dosage of 100 µg/L Na-acetate. Error bars represent 95 % CI.

5.5.2.2.2 Biofilm

At a dosage of 100 µg/L Na-acetate *P. aeruginosa* was detected once in Bs 1 and Bs 3, respectively, eight weeks after first artificial contamination at an operating temperature of 36°C but never at 20°C operating temperature (Table 21).

E. faecalis was detected once in Bs 1 four weeks after first artificial contamination and twice in Bs 2 after four and eight weeks after first artificial contamination at a dosage of 100 µg/L Na-acetate and an operating temperature of 20°C. At the elevated operating temperature *E. faecalis* was only once detected four weeks after first artificial contamination in Bs 1 and Bs 2, respectively (Table 21).

E. coli was never detected in any of the biofilm sections (Table 21).

None of the contaminants were detected in Bs 4 (Table 21), neither at 20°C nor at 36°C operating temperature. These observations indicate that the CAC-filter acted as a barrier.

Table 21: Colony counts of contaminants in the biofilm sections of Rig Con at a dosage of 100 µg/L Na-acetate and operating temperatures of 20°C and 36°C. Numbers in brackets represent the week of detection after first artificial contamination.

	20°C, 100 µg/L Na-acetate (TS 5)				**36°C, 100 µg/L Na-acetate (TS 3)**			
	Bs 1	**Bs 2**	**Bs 3**	**Bs 4**	**Bs 1**	**Bs 2**	**Bs 3**	**Bs 4**
	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2
P. aeruginosa	< 0,06	< 0,06	< 0,06	< 0,06	0,04 (8)	< 0,06	0,04 (8)	< 0,06
E. faecalis	0,06 (4)	0,06 (4),(8)	< 0,06	< 0,06	0,16 (4)	0,16 (4)	< 0,06	< 0,06
E. coli	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06

Analysis with FISH revealed only one case of suspected *P. aeruginosa* in Bs 2 at 36°C operating temperature and with a dosage of 100 µg/L Na-acetate four weeks after first artificial contamination (Figure 22). This indicates that beside *E. faecalis, P. aeruginosa* contributed to the retrograde contamination four weeks after first artificial contamination (Table 21). Because *P. aeruginosa* was not detected by the cultivation method in the same sample, *P. aeruginosa* might have been present in a VBNC state. If a bacterium enters the VBNC state it remains its reproductive capacity but cannot be detected with standard methods, which require a cultivation step (Oliver 2005). Dwidjosiswojo et al. (2011) identified Cu as initiator for the VBNC state of *P. aeruginosa* in suspension starting from a concentration of 0,1 µmol of Cu in solution. Since the fill-up water contained about 1,1 µmol Cu (Table 13) the VBNC-state of *P. aeruginosa* could have been initialized by this cation. Against the hypothesis that *P. aeruginosa* might has entered the VBNC-state initialized by Cu, is the fact that *P. aeruginosa* was detected culturably in water and biofilm samples.

At 36°C operating temperature and with a dosage of 100 µg/L Na-acetate *P. aeruginosa* was not detected with FISH in the biofilm sections Bs 1, Bs 3 and Bs 4 of Rig Con and not in any biofilm section of Rig Ref (TS 3) (data not shown).

P. aeruginosa was not detected with FISH at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 5) (data not shown).

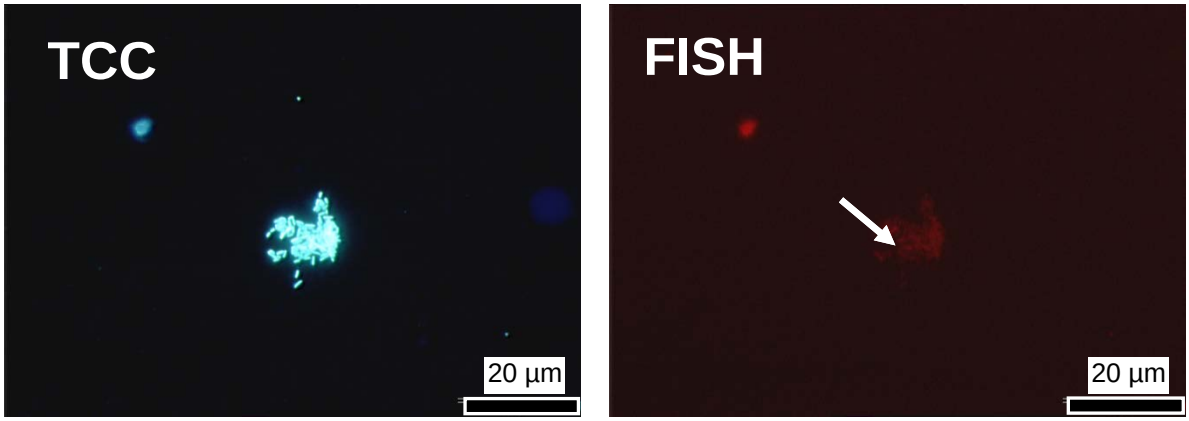

Figure 22: Microscopic pictures of biofilm taken from biofilm section Bs 2 of Rig Con at operation week 10, four weeks after first artificial contamination of TS 3 (36°C, 100 µg/L Na-acetate). DAPI stained bacteria (left) and cells hybridized with probe Psae16S-182 (right) from the identical microscopic field.

Results of contaminants at a dosage of 100 µg/L Na-acetate can be summarized as follows:

At a dosage of 100 µg/L Na-acetate a retrograde contamination occurred at 20°C and 36°C operating temperature but was at 20°C operating temperature limited to the biofilm.

A systemic contamination of the biofilms and a strong proliferation of the contaminants inside the tank water did not occur. This finding support the results discussed at the end

of chapter 5.4.2 where the faucet seemed to the favored habitat of *P. aeruginosa* and *E. faecalis* within the MDWS. The low concentration of *P. aeruginosa* and *E. faecalis* in the tank water and the biofilm sections Bs 1, Bs 2 and Bs 3 might be explained by the presence of well adapted autochthonous bacteria, usage of proper type of material in contact with water or specific water constituents as hypothesized and discussed in chapter 5.4.2.1.

Since no cultivation independent method like FISH had been applied for the detection of *E. faecalis* and *E. coli*, the bacteria might be present in a VBNC state, even at high concentrations, as demonstrated for *E. coli* in biofilms by Juhna et al. (2007).

5.5.3 Impact of temperature at high OC-concentration

5.5.3.1 Total cell counts and colony counts

5.5.3.1.1 Water

Tank water

Combination of a dosage of 300 µg/L Na-acetate and an operating temperature of 36°C had neither an effect on the CC 20°C of the water phase nor on the CC 20°C of the biofilm samples (data not shown).

Similar to the results obtained for a dosage of 100 µg/L Na-acetate (chapter 5.5.2, Figure 17 a), the CC 36°C of the tank water at 300 µg/L was about 2 log-cycles higher compared to 20°C operating temperature (Figure 23 a). The increase was so pronounced that the CC 36°C exceeded the limiting value of 100 cfu/mL (TrinkwV 2001) in the vast majority of all samples (Figure 23 a). Similar to the results found for the elevated OC-concentration (chapter 5.5.2.1.1) the high OC-concentration seemed to sustain culturability. It is worth mentioning again, that sustentation of culturability could not be validated because no cultivation independent method like TCC had been applied for water samples. In accordance with the CC 36°C, the HPC was higher at the elevated operating temperature but in contrast to the CC 36°C it increased only 1 log-cycle (Figure 23 a and b).

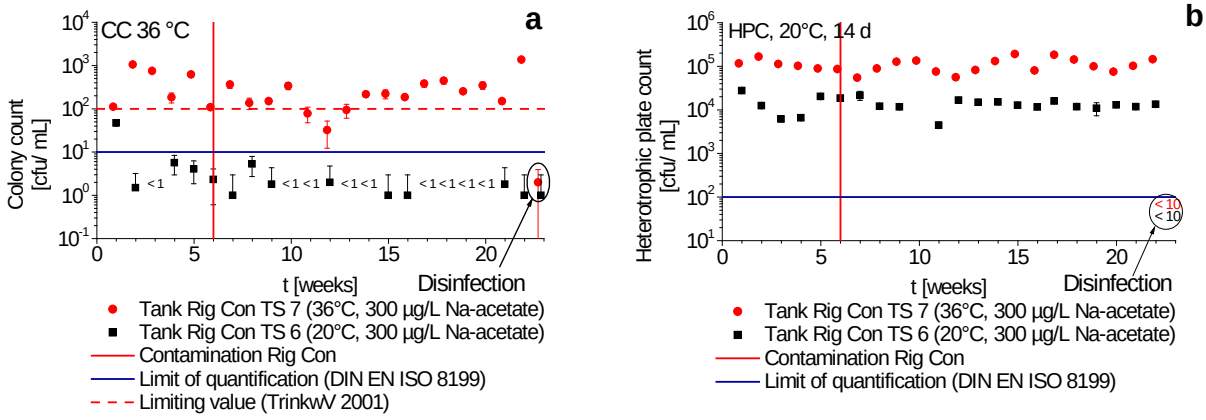

Figure 23: CC 36°C (a) and HPC (b) of tank water from Rig Con at low (TS 6) and elevated (TS 7) operating temperature always with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

Water from spigot

With a dosage of 300 µg/L Na-acetate and at an elevated operating temperature of 36°C the CC 36°C of the spigot revealed varying results but in general higher counts than at 20°C operating temperature. Although the CC 36°C increased due to an increased operating temperature the values remained below the limiting value of 100 cfu/mL (TrinkwV 2001) after 9 weeks of operation (Figure 24 a). Similar to the CC 36°C, the HPC from the spigot varied but was in general higher at the elevated operating temperature (Figure 24 b). These results were similar to the results with dosage of 100 µg/L Na-acetate (chapter 5.5.2, Figure 18). Although the HPC of the tank water increased due to an elevated operating temperature the CAC-filter reduced the HPC by more than 1 log-cycle or 90 % (Figure 23 b, Figure 24 b).

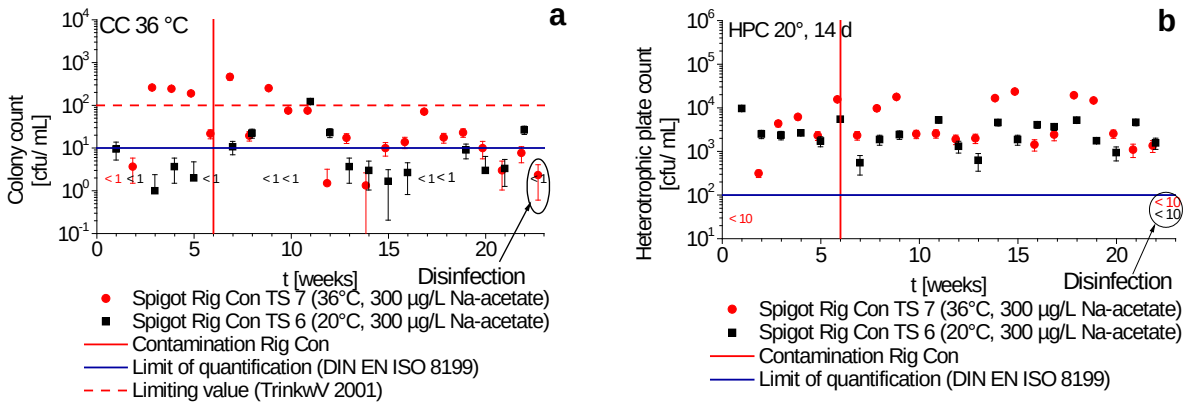

Figure 24: CC 36°C (a) and HPC (b) of water taken from the spigot of Rig Con at low (TS 6) and elevated (TS 7) operating temperature always with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

Water from the faucet

Similar to water samples from the tank (Figure 23 a), the CC 36°C was increased at the faucet due to the elevated operating temperature of 36°C and exceeded the limiting value of 100 cfu/mL (TrinkwV 2001) in the vast majority of all samples (Figure 25 a). In comparison to the lower operating temperature the CC 36°C was until 15th week of operation in general 1 to 2 log-cycles and later at least 2 log-cycles higher at elevated operating temperature. Similar to the tank water (Figure 23 a) a high OC-concentration at elevated operating temperature seemed to sustain culturability. Except for the first week of operation the HPC was constantly about 1 log-cycle higher at elevated operating temperature (Figure 25 b). These results were similar to the results with dosage of 100 µg/L Na-acetate (chapter 5.5.2, Figure 19 b).

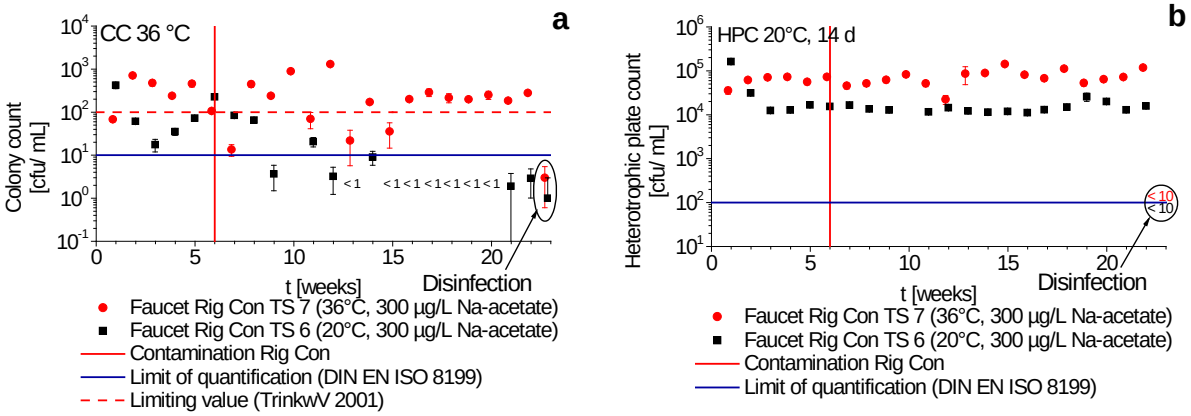

Figure 25: CC 36°C (a) and HPC (b) of water taken from the faucet of Rig Con at low (TS 6) and elevated (TS 7) operating temperature with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

5.5.3.1.2 Biofilm

With a dosage of 300 µg/L Na-acetate, the elevated operating temperature increased the CC 36°C of Bs 1, Bs 2, and Bs 3 until about the 10th week of operation, afterwards it decreased to the level of the low operating temperature (Figure 26 a, b and c). In Bs 1 and Bs 2 the temperature-dependent increase was most pronounced. From the first sampling in operation week 2, the CC 36°C was 1 to 2 log-cycles higher compared to the operating temperature of 20°C (Figure 26 a and b). The temperature-dependent increase of CC 36°C was in Bs 3 less pronounced and developed about 3 weeks later than in Bs 1 and Bs 2 (Figure 26 c). The vast majority of all samples in Bs 4 had values of CC 36°C which were below or close to the quantification limit, making interpretation of results insecure. The increased operating temperature of 36°C seemed to increase the CC 36°C of Bs 4 until about the 5th week of operation, afterwards it decreased to the level of low operating temperature (Figure 26 d).

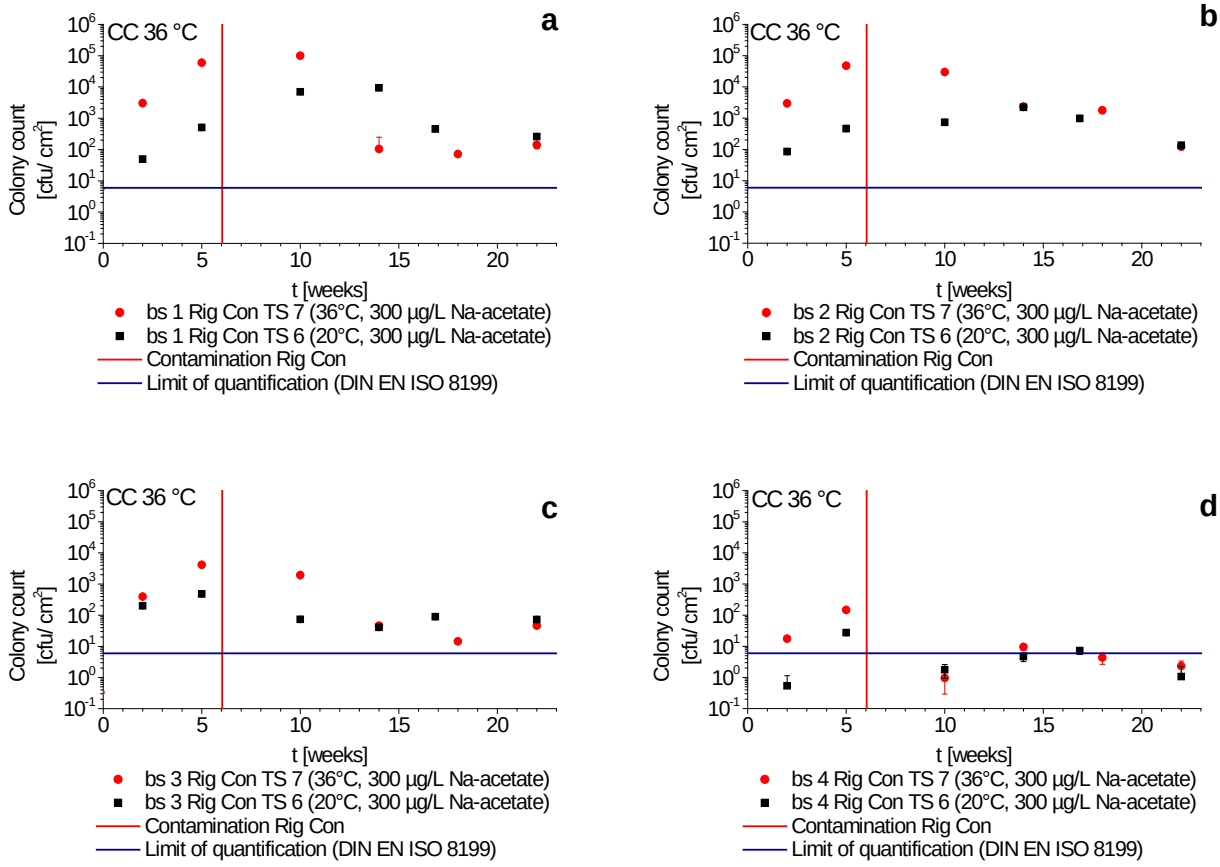

Figure 26: CC 36°C of biofilm from Bs 1 (a), Bs 2 (b), Bs 3 (c) and Bs 4 (d) of Rig Con at low (TS 6) and elevated (TS 7) operating temperature with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

With a dosage of 300 µg/L Na-acetate the HPC of Bs 1, Bs 2, Bs 3, and Bs 4 was not influenced by the elevated operating temperature of 36°C compared to 20°C (data not shown). This also implies that the CAC-filter reduced the biofilm formation in Bs 4 even at a very high OC-concentration. Similar to the HPC, the TCC of Bs 1, Bs 2, and Bs 3 was temperature independent at a dosage of 300 µg/L Na-acetate (data not shown). In contrast to all other biofilm sections, the TCC in Bs 4 decreased after 14 weeks of operation at the elevated operating temperature (Figure 27).

Since the TCC remained constant in the biofilm sections Bs 1, Bs 2, Bs 3 and until operation week 14 in Bs 4 and the CC 36°C decreased already from week 10 in the biofilm sections Bs 1, Bs 2, Bs 3 and from week 5 in Bs 4, the bacteria lost culturability. This finding is similar to the results found for the biofilms at elevated OC-concentration (chapter 5.5.2.1.2). The loss of culturability of biofilm bacteria, while possibly sustaining culturability of planktonic bacteria (chapter 5.5.3.1.1) at high OC-concentrations, is similar to the results found for the elevated OC-concentration (chapter 5.5.2.1.1 and 5.5.2.1.2).

The decrease of TCC in Bs 4 after a certain time of operation indicates that the filtration quality of the CAC-filter increased with operation time. The increased filtration quality could be explained by an increasing colonization of the CAC-filter with bacteria, which enhanced the filtration quality due to their metabolism. At the same time the CAC-filter had to be a barrier for those bacteria.

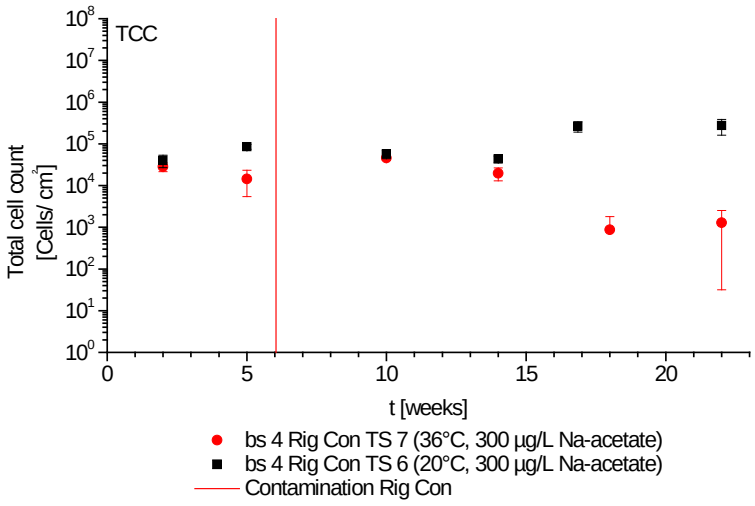

Figure 27: TCC of the biofilm from Bs 4 of Rig Con at low (TS 6) and elevated (TS 7) operating temperature always with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

Since the increase in CC 36°C in Bs 3 was less pronounced compared to Bs 2 and both biofilm sections had the same distance to the acetate dosage (Figure 1 and Table 3), it was concluded that the higher flow through of Bs 2 led to a better nutrient supply of its biofilm. Besides the better nutrient supply, the mean temperature of Bs 2 had to be higher compared to Bs 3, since more than twice of the water volume flowed through Bs 2.

5.5.3.2 Facultative pathogens

5.5.3.2.1 Water

Water from tank and spigot

During the test series with a dosage of 300 µg/L Na-acetate (TS 6 and TS 7) none of the contaminants were detected in water taken from tank and spigot.

Water from faucet

In contrast to the test series with 100 µg/L Na-acetate (chapter 5.5.2, Figure 21 a) the concentration of *P. aeruginosa* did not increase with operating temperature when operated with 300 µg/L Na-acetate until the 16th week of operation (Figure 28 a). The comparably low concentration of *P. aeruginosa* at the faucet might be the reason why no retrograde contamination occurred in the tank water. From week 16 on, the concentration of *P. aeruginosa* varied and increased finally by 1 to 2 log-cycles at elevated operating temperature (Figure 28 a). *P. aeruginosa* was not detected from operation week 16 on at the lower operating temperature of 20°C (Figure 28 a).

In summary, at both operating temperatures (20°C and 36°C) the lower OC-concentration (100 µg/L Na-acetate) revealed a higher concentration of *P. aeruginosa*, especially at the elevated operating temperature (Figure 21 a and Figure 28 a).

With a dosage of 300 µg/L Na-acetate and at an elevated operating temperature of 36°C *E. faecalis* was detected more often and in general at higher concentrations compared to the lower operating temperature of 20°C (Figure 28 b).

The contaminant *E. coli* was never detected in water samples from the faucet (data not shown).

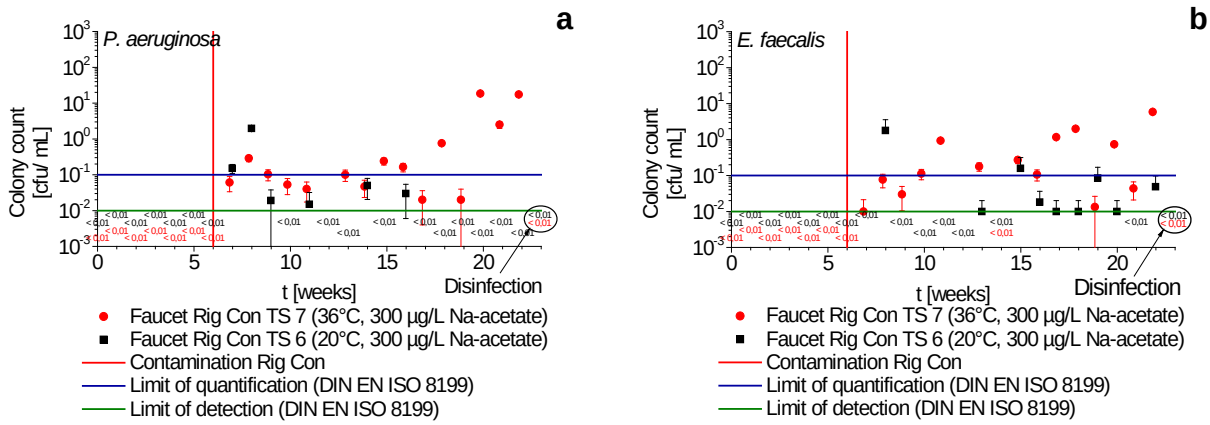

Figure 28: Colony counts of *P. aeruginosa* (a) and *E. faecalis* (b) of water taken from the faucet of Rig Con at low (TS 6) and elevated (TS 7) operating temperature with dosage of 300 µg/L Na-acetate. Error bars represent 95 % CI.

In summary, the concentration of *P. aeruginosa* and *E. faecalis* in water phase was influenced by the concentration of organic carbon and operating temperature but was not directly correlated to the parameters.

Regarding the findings of the present work and the findings of van der Kooij et al. (1982), Moreira et al. (1994), Moritz et al. (2010) and Benölken (2010), the culturable concentration of *P. aeruginosa* in drinking water does not solely depend on single parameters. Up to now it seems to be a more complex interaction including water temperature, type of material in contact with water, concentration and composition of substrate, competition with autochthonous bacteria for substrate and interaction of planktonic bacteria and biofilm bacteria.

5.5.3.2.2 Biofilm

P. aeruginosa was detected once in Bs 1, Bs 2, and Bs 3, respectively, four weeks after first artificial contamination at 20°C operating temperature and a dosage of 300 µg/L Na-acetate. In contrast to the results at low operating temperature, *P. aeruginosa* was not detected at elevated operating temperature (Table 22).

E. faecalis was detected twice in Bs 2 with a dosage of 300 µg/L Na-acetate and at an operating temperature of 20°C. First detection had been 11 weeks and second detection 16 weeks after first artificial contamination. At elevated operating temperature *E. faecalis* was again detected twice in Bs 2 and additionally twice in Bs 1. The detection in Bs 2 at elevated operating temperature had been 3 weeks earlier compared to the lower operating temperature (Table 22).

The test series with the combination of the elevated operating temperature and dosage of 300 µg/L Na-acetate was the only combination that led to the detection of *E. coli* in biofilms. *E. coli* was detected once in Bs 1 eight weeks after first artificial contamination and a second time in Bs 2 after 16 weeks of operation since first artificial contamination.

Table 22: Colony counts of contaminants in the biofilm sections of Rig Con with a dosage of 300 µg/L Na-acetate and operating temperatures of 20°C and 36°C. Numbers in brackets represent the week of detection after first artificial contamination.

	20°C, 300 µg/L Na-acetate (TS 6)				**36°C, 300 µg/L Na-acetate (TS 7)**			
	Bs 1	**Bs 2**	**Bs 3**	**Bs 4**	**Bs 1**	**Bs 2**	**Bs 3**	**Bs 4**
	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2	cfu/cm^2
P. aeruginosa	23,25 (4)	0,86 (4)	0,08 (4)	< 0,06	< 0,06	< 0,06	< 0,06	< 0,06
E. faecalis	< 0,06	0,06 (11) 0,06 (16)	< 0,06	< 0,06	0,17 (8) 0,06 (12)	0,24 (8) 0,06 (12)	< 0,06	< 0,06
E. coli	< 0,06	< 0,06	< 0,06	< 0,06	0,06 (8)	0,05 (16)	< 0,06	< 0,06

Analysis with FISH resulted in a detection of *P. aeruginosa* in Bs 1 at 20°C operating temperature and a dosage of 300 µg/L Na-acetate four weeks after artificial contamination, supporting the results from cultivation method as already shown and discussed in chapter 5.4.2.2.

Identical to the results from cultivation method (Table 22), *P. aeruginosa* was not detected with FISH at 36°C operating temperature and a dosage of 300 µg/L Na-acetate (data not shown).

Results of contaminants at a dosage of 300 µg/L Na-acetate can be summarized as follows:

With a dosage of 300 µg/L Na-acetate a retrograde contamination occurred at 20°C and 36°C but was limited to the biofilm. Only the combination of the elevated operating temperature and a dosage of 300 µg/L Na-acetate led to a retrograde contamination by *E. coli*.

With a dosage of 300 µg/L Na-acetate a systemic contamination of the biofilms or the water did not occur.

Similar to all operating conditions with artificial contamination (chapters 5.4 and 5.5), the faucet seemed to be the favored habitat of *P. aeruginosa* and *E. faecalis* within the MDWS.

The low concentration and seldom occurrence of *P. aeruginosa* and *E. faecalis* in the biofilm sections Bs 1, Bs 2 and Bs 3 might be explained by the presence of well adapted autochthonous bacteria, proper type of material in contact with water or specific water constituents as hypothesized and discussed in chapter 5.4.2.1.

Since no cultivation independent method like FISH had been applied for the detection of *E. faecalis* and *E. coli*, the bacteria might be present in a VBNC state, even at high concentrations, as demonstrated for *E. coli* in biofilms by Juhna et al. (2007).

5.6 Evaluation of disinfection procedures

Disinfection procedure 1 (two-step disinfection with H_2O_2, see chapter 4.4) had been applied after TS 1 had been completed. After 2 weeks of operation of TS 2 *P. aeruginosa* was detected in water taken from the faucet, demonstrating that the disinfection procedure had been insufficient.

Disinfection procedure 2 (single-step disinfection with NaOCl) had been applied after TS 2 and TS 3 had been completed. During biofilm establishment of TS 3 no contaminant occurred, indicating that the disinfection procedure 2 was sufficient. During biofilm establishment of TS 4 *P. aeruginosa* was detected 4 weeks after disinfection in water taken from the faucet demonstrating that the disinfection procedure 2 did not disinfect Rig Con completely after finishing TS 3.

In order to ensure a secure inactivation of all contaminants, the CTC-disinfection (NaOCl → 70°C, 3 min → NaOCl) had been applied after abortion of TS 4. The CTC-disinfection had also been applied after TS 5, TS 6, and TS 7. During biofilm establishment of TS 5, TS 6, and TS 7 none of the contaminants were detected, which indicates that the CTC-disinfection is a safe disinfection procedure.

According to this, van der Mee-Marquet et al. (2005) reported a successful eradication of *P. aeruginosa* by intensive chlorination of a central water distribution system of a newly built wing of a hospital, while several non-touch faucets remained positive for *P. aeruginosa*. After chlorination, they conducted a thermal disinfection which consisted of a continuous water flow at each tap for 30 min with a water temperature of 70°C. After this treatment all water samples remained negative for *P. aeruginosa* over the entire investigation period of six month.

6 Conclusions

Each of the facultative pathogenic bacteria *E. faecalis*, *P. aeruginosa* and *E. coli* carries the potential to contaminate retrogradely MDWS starting from a terminal faucet. At each tested boundary condition a retrograde contamination developed. Retrograde contaminations occurred in biofilms and tank water and consisted mostly of *P. aeruginosa* and *E. faecalis*. This is in accordance with the findings of a study conducted by APHA in 1999, cited in WHO's 3rd edition of 'Guide to Hygiene and Sanitation in Aviation' (WHO 2009), where enterococci and especially *P. aeruginosa* were detected much more frequently in water samples taken from mains, bowsers and aircraft than *E. coli*.

Even though all tested bacteria were capable of contaminating retrogradely MDWS, and were able to persist for at least up to four months in high concentrations at the faucet, they did not colonize systemically the MDWS. From the experimental results that the contamination remained rather local at the faucet and the findings of a study conducted by Health Canada in 2006 (WHO 2009) that most contaminations were found in water from lavatory faucets, it can be concluded that the retrograde contamination starting from a lavatory faucet is probably a common path for microbial contamination in MDWS.

7 Recommendations

Measures to reduce the risk of retrograde contaminations of MDWS can be subdivided into three major targets:

1. Prevention of contamination

- Design improvement of terminal faucets could prevent a retrograde contamination. If the water outlet would be recessed into the faucet, a contact contamination, e.g. by unwashed hands, would be prevented.
- Use of terminal sterile filters as applied in hospitals with contaminated drinking water systems in order to protect the consumer from pathogens in water (e.g. *Legionella pneumophila*; Vonberg et al., 2005). The sterile filters can also be used to protect the drinking water system from pathogens coming with contaminations from outside. If terminal filters will be applied they have to be installed without any bypass in order to prevent a retrograde contamination. It has to be mentioned that once a filter is contaminated from outside, it might deliver contaminated water, which makes regular exchange of filters mandatory.

2. Reduction in spread of contamination

- Regular cleaning and disinfection of galleys and sanitary units, especially faucets and its aerators.
- Bunkering of safe and high quality water, i.e. the water has to be free of pathogens, cold and as oligotrophic as possible.
- Cold storage and distribution of bunkered water. Therefore tanks and water distribution pipes should be installed in locations where warming up of the water is prevented. If this is not possible tanks and pipes have to be thermally insulated.
- A CAC-filter reduces the risk of retrograde contamination originating from consumption points outside the section with the installed filter.
- Continuous disinfection of tank water or bunkering of water containing disinfectant residual. If continuous disinfection is applied four important aspects have to be considered:

  1. In the presence of natural organic matter the usage of chlorine as disinfectant leads to the development of carcinogenic trihalomethanes (limiting values have to be considered).
  2. For hypochlorite the pH-value has to be adjusted and kept below pH 8 in order to ensure safe disinfection.
  3. A sufficient disinfectant residual at the most distant consumption point at any time is mandatory.
  4. Once drinking water disinfection is applied, continuity of disinfection becomes mandatory. Continuity is necessary because the oxidation of non-biodegradable organic carbon by the disinfectant could result in biodegradable carbon, which could support strong proliferation of bacteria including pathogens.

  Further details on disinfection of drinking water can be found in WHO (2008) and in DVGW W 290.

- Removal of biodegradable residues from production of parts of the drinking water system, like e.g. drawing grease on pipe surfaces. Information on degreasing removal can be found in DIN 65 473 and DIN EN 2516.
- Usage of material and auxiliary materials that do not deteriorate drinking water quality.

  Further information on suitable materials in contact with drinking water can be found in DIN 1988-200 and DIN EN 806-2. For organic materials in contact with drinking water, the German Federal Environmental Agency “Umweltbundesamt”

published the Hose Line Recommendation (2002) and following guide lines: Coating Guideline (2011), KTW Guideline (2009), Lubricant Guideline (2011) and Elastomer Guideline (2011).

Further details on drinking water quality and maintenance of drinking water quality can be found in WHO (2008). Additionally, there are WHO-guides specified for hygiene and sanitation aboard ships (WHO 2007) and in aviation (WHO 2009).

3. Elimination of contamination

- If all precautionary measures and countermeasures against a retrograde contamination failed, a thorough and lasting disinfection of the potable water system will be necessary. A suitable procedure for the tested conditions is the CTC-disinfection as described in this report (chapter 4.4). Previous to any disinfection procedure the system should be cleaned mechanically.

Further details on disinfection of drinking water systems can be found in DVGW W 291 and DVGW W 551.

8 Summary

The facultative pathogenic bacteria *E. faecalis*, *P. aeruginosa* and *E. coli* were tested for their ability to contaminate retrogradely mobile drinking water systems. Therefore two identical test rigs were installed and operated. One represented a reference, the other was artificially contaminated with the pathogens at a terminal consumption point. The fill-up water quality was varied in terms of nutrient concentration and temperature, so that finally the occurrence of a retrograde contamination can be assessed regarding these two main factors. Water and biofilm samples were taken regularly from characteristic points and were analysed for total cell count, colony count and for the facultative pathogens according to international standards. Additionally, fluorescence in situ hybridization was applied for detection of *P. aeruginosa*.

In general it can be stated that at an operating temperature of 20°C without addition of acetate the microbiological data of the water quality were highly reproducible (chapter 5.2). The CC 20°C turned out to be an inadequate parameter for the assessment of the microbiological quality of both biofilm and drinking water of MDWS.

Core results of the present work can be summarized as follows:

Drawing grease supports biofilm formation

Drawing grease is a residue from pipe manufacturing and supports biofilm formation. This can be observed in water with a low organic carbon concentration. A high CC 36°C in new pipes from the beginning on might be a sensitive parameter for the presence of drawing grease on pipe surfaces (chapter 5.2).

Biofilm formation depends on flow through

Naturally occurring DOC and especially dosed acetate support biofilm formation. Additionally, it can be stated that the nutrient supply and consequently biofilm formation increased with increasing water flow-through (chapter 5.2).

Elevated OC-concentrations result in an elevated HPC

Higher concentrations of easily assimilable OC at an operating temperature of 20°C significantly increase the HPC of water and biofilm samples but not the standard parameters CC 20°C and CC 36°C (chapter 5.4.1).

Elevated operating temperature result in an elevated CC 36°C

Higher operating temperatures of 36°C significantly increase the CC 36°C of water samples, possibly above the limiting value of TrinkwV 2001 (chapters 5.5.1.1.1, 5.5.2.1.1 and 5.5.3.1.1).

Elevated operating temperature has little influence on biofilm

The elevated operating temperature alone has only little influence on colony counts (HPC, CC 36°C) of the biofilm. However, this result is based on experiments without thermally insulated pipes. Therefore, a difference of about 16°C water temperature could not be maintained in the pipes further away from the tank. This might be the reason for similar results for originally different boundary conditions. Additionally, bacteria from biofilm loose culturability with operation time (chapters 5.5.1.1.2, 5.5.2.1.2 and 5.5.3.1.2).

***P. aeruginosa* and *E. faecalis* persist at the faucet**

P. aeruginosa and *E. faecalis* have the ability to persist at the faucet, whereas *E. coli* is not able to persist at the faucet (Table 23 and chapters 5.4.2.1, 5.5.2.2.1, 5.5.3.2.1). An operating temperature of 20°C combined with a high OC-concentration (dosage of 300 µg/L Na-acetate) lowered the concentration of *P. aeruginosa* and *E. faecalis* at the faucet, possibly due to an increased number of other bacteria competing for substrate (chapter 5.4.2.1). An elevated OC-concentration (dosage of 100 µg/L Na-acetate) combined with an elevated operating temperature supported the growth of *P. aeruginosa* at the faucet (Table 23 and chapter 5.5.2.2.1).

Retrograde contamination of water phase

An elevated OC-concentration (dosage of 100 µg/L Na-acetate) combined with the elevated operating temperature of 36°C was the only combination of boundary conditions where a retrograde contamination with *P. aeruginosa* and *E. faecalis* of the tank water occurred (Table 23 and chapter 5.5.2.2.1).

Table 23: Overview of detected contaminants in water samples taken from Rig Con. Red crosses indicate retrograde contamination.

T [°C]	**Na-acetate** [µg/L]	**Sampling point**	***P. aeruginosa***	***E. faecalis***	***E. coli***
20	**100**	Faucet	++	++	-
		Tank	-	-	-
		Spigot	-	-	-
	300	Faucet	++	++	-
		Tank	-	-	-
		Spigot	-	-	-
36	**100**	Faucet	+++	++	-
		Tank	**+**	**+**	-
		Spigot	-	-	-
	300	Faucet	++	++	-
		Tank	-	-	-
		Spigot	-	-	-

- < 0,01 cfu/mL; + 0,01 - 1 cfu/mL; ++ 1 – 100 cfu/mL; +++ > 100 cfu/mL

Retrograde contamination of biofilm

Every combination of applied boundary conditions resulted in a retrograde contamination of the biofilms by either *P. aeruginosa* or *E. faecalis* or both (Table 24 and chapters 5.4.2.2, 5.5.2.2.2, 5.5.3.2.2). Only the combination of elevated operating temperature and high OC-concentration (dosage of 300 µg/L Na-acetate) resulted in a retrograde contamination by *E. coli* (Table 24 and chapter 5.5.3.2.2).

Table 24: Overview of detected contaminants in biofilm samples taken from Rig Con. Red crosses indicate retrograde contamination.

T [°C]	Na-acetate [µg/L]	Sampling point	*P. aeruginosa*	*E. faecalis*	*E. coli*
20	**100**	Bs 1 (downstream Tank)	-	+	-
		Bs 2 (Tank ←→Toilet)	-	+	-
		Bs 3 (Galley previous to CAC)	-	-	-
		Bs 4 (Galley downstream CAC)	-	-	-
	300	Bs 1 (downstream Tank)	+++	-	-
		Bs 2 (Tank ←→Toilet)	++	+	-
		Bs 3 (Galley previous to CAC)	+	-	-
		Bs 4 (Galley downstream CAC)	-	-	-
36	**100**	Bs 1 (downstream Tank)	+	++	-
		Bs 2 (Tank ←→Toilet)	-	++	-
		Bs 3 (Galley previous to CAC)	+	-	-
		Bs 4 (Galley downstream CAC)	-	-	-
	300	Bs 1 (downstream Tank)	-	++	-
		Bs 2 (Tank ←→Toilet)	-	++	+
		Bs 3 (Galley previous to CAC)	-	-	-
		Bs 4 (Galley downstream CAC)	-	-	-

- < 0,06 cfu/cm^2; + 0,06 – 0,1 cfu/cm^2; ++ 0,1 – 1 cfu/cm^2; +++ > 1 cfu/cm^2

No strong proliferation of contaminants inside the MDWS

Since the detection of any retrograde contamination in biofilms and water had been close to the corresponding detection limit and since the concentration of contaminants did not increase with time of operation, it was concluded that there is no strong proliferation of contaminants in the system (chapters 5.4.2, 5.5.2.2 and 5.5.3.2).

CAC-filter removes DOC and bacteria

At any tested operating condition the CAC-filter was able to remove planktonic bacteria and DOC from water over 22 weeks of operation, resulting in a reduced concentration of planktonic bacteria and a reduced biofilm formation downstream the filter (chapter 5.2).

None of the contaminants were ever detected downstream the CAC-filter, indicating that the filter acts as a barrier for bacteria (chapters 5.4.2, 5.5.2.2 and 5.5.3.2).

CTC-disinfection is a successful procedure

The CTC-disinfection (NaOCl → 70°C, 3 min → NaOCl) proved to be a secure and lasting disinfection procedure for MDWS contaminated with facultative pathogenic bacteria (chapter 5.6).

Measures against retrograde contamination

The occurrence of a retrograde contamination by experimentally added facultative pathogenic bacteria at every tested combination of boundary conditions was demonstrated in the present work. Based on that finding, it is recommended to apply a multi target concept in order to prevent retrograde contaminations. It consists of three major targets. The first target is the prevention of contamination, e.g. by a device protecting the aerator from touching with unwashed hands. The second target is the reduction in spread of contamination by maintaining high water quality and regular cleaning and disinfection of consumption points and their periphery. The third target is the elimination of pathogens from contaminated MDWS by cleaning and a safe disinfection procedure (e.g. CTC-disinfection, (chapter 7).

9 Literature

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10 Appendix

10.1 Raw data of TS 1

Water samples – Rig Ref

Table A 1: Raw data of tank water taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 1)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,6	< 0,01		< 0,01		< 0,01		< 1		17	4	720	96
4,6	< 0,01		< 0,01		< 0,01		10	3	12	4	2,0E+03	152
5,6	< 0,01		< 0,01		< 0,01		8	3	6	3	808	97
6,6	< 0,01		< 0,01		< 0,01		14	4	16	4	1,1E+03	112
7,6	< 0,01		< 0,01		< 0,01		8	3	25	5	928	103
8,6	< 0,01		< 0,01		< 0,01		< 1		< 1		1,1E+03	113
9,6	< 0,01		< 0,01		< 0,01		4	2	3	2	1,8E+03	146
10,6	< 0,01		< 0,01		< 0,01		< 1		10	4	1,4E+03	126
11,6	< 0,01		< 0,01		< 0,01		5	3	11	4	1,3E+03	122
12,6	< 0,01		< 0,01		< 0,01		2	2	9	3	1,8E+03	142
13,6	< 0,01		< 0,01		< 0,01		8	4	2	2	1,6E+03	137
14,6	< 0,01		< 0,01		< 0,01		5	4	2	1	1,2E+03	115
15,6	< 0,01		< 0,01		< 0,01		19	5	9	3	1,2E+03	116
16,6	< 0,01		< 0,01		< 0,01		5	4	23	5	1,2E+03	141
17,6	< 0,01		< 0,01		< 0,01		< 1		< 1		1,3E+03	122
18,6	< 0,01		< 0,01		< 0,01		29	6	3	3	261	55
19,6	< 0,01		< 0,01		< 0,01		10	20	7	5	2,8E+03	178
20,6	< 0,01		< 0,01		< 0,01		20	28	5	2	1,7E+03	138
21,7	< 0,01		< 0,01		< 0,01		< 1		< 1		601	84
24,6	< 0,01		< 0,01		< 0,01		3	2	88	11	2,8E+03	289

Water samples – Rig Con

Table A 2: Raw data of tank water taken from Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,6	< 0,01		< 0,01		< 0,01		< 1		63	9	2,7E+03	322
4,6	< 0,01		< 0,01		< 0,01		13	4	7	3	1,6E+03	136
5,6	< 0,01		< 0,01		< 0,01		6	3	7	5	1,9E+03	148
6,6	0,30	0,11	1,00	0,20	1,88	0,26	22	5	31	6	2,3E+03	162
7,6	0,03	0,01	< 0,01		0,01	0,01	7	3	9	4	1,0E+04	1,1E+03
8,6	< 0,01		< 0,01		< 0,01		< 1		20	5	8,0E+03	967
9,6	< 0,01		< 0,01		< 0,01		23	5	3	2	1,6E+04	1,4E+03
10,6	< 0,01		< 0,01		< 0,01		11	4	35	6	1,4E+04	1,3E+03
11,6	< 0,01		< 0,01		< 0,01		8	3	25	5	1,9E+04	1,5E+03
12,6	< 0,01		< 0,01		< 0,01		14	4	20	6	2,4E+04	1,7E+03
13,6	< 0,01		< 0,01		< 0,01		13	4	9	3	2,3E+04	1,6E+03
14,6	< 0,01		< 0,01		< 0,01		2	2	44	7	1,4E+04	1,3E+03
15,6	< 0,01		< 0,01		< 0,01		28	6	48	7	2,1E+04	1,6E+03
16,6	< 0,01		< 0,01		< 0,01		2	2	31	6	2,5E+03	171
17,6	< 0,01		< 0,01		< 0,01		< 1		< 1		1,4E+03	129
18,6	< 0,01		< 0,01		< 0,01		17	5	190	15	1,3E+03	123
19,6	< 0,01		< 0,01		< 0,01		19	8	252	27	1,1E+04	1,1E+03
20,6	< 0,01		< 0,01		< 0,01		12	4	23	5	1,4E+04	1,3E+03
21,7	< 0,01		< 0,01		< 0,01		< 1		< 1		109	44
24,6	< 0,01		< 0,01		< 0,01		7	4	tntc	tntc	3,9E+04	2,2E+03

Table A 3: Raw data of taken from the faucet of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa* WAM	± 95 % CI	*E. coli* WAM	± 95 % CI	*E. faecalis* WAM	± 95 % CI	CC 20°C WAM	± 95 % CI	CC 36°C WAM	± 95 % CI	HPC WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,6	< 0,01		< 0,01		< 0,01		n. d.		n. d.		n. d.	
4,6	< 0,01		< 0,01		< 0,01		n. d.		n. d.		n. d.	
5,6	< 0,01		< 0,01		< 0,01		n. d.		n. d.		n. d.	
6,6	590,00	150,55	15,10	2,41	31,90	11,07	688	89	827	98	3,7E+03	328
7,6	0,77	0,17	< 0,01		< 0,01		30	6	334	31	1,4E+04	1,3E+03
8,6	1,88	0,26	0,03	0,01	< 0,01		< 1		86	10	2,6E+04	1,7E+03
9,6	1,18	0,21	0,01	0,01	< 0,01		15	4	47	7	9,0E+03	1,0E+03
10,6	0,37	0,08	< 0,01		< 0,01		35	26	231	16	3,3E+04	3,1E+03
11,6	0,22	0,09	< 0,01		< 0,01		< 1		161	14	2,9E+04	1,8E+03
12,6	0,16	0,08	< 0,01		< 0,01		3	2	76	9	3,1E+04	6,0E+03
13,6	0,13	0,07	< 0,01		< 0,01		18	5	21	5	8,2E+04	9,8E+03
14,6	0,07	0,02	< 0,01		< 0,01		12	4	102	11	2,8E+04	1,8E+03
15,6	0,08	0,02	< 0,01		< 0,01		88	10	104	11	6,9E+04	9,1E+03
16,6	0,30	0,11	< 0,01		< 0,01		3	2	155	13	2,3E+03	518
17,6	0,04	0,01	< 0,01		< 0,01		< 1		< 1		3,3E+03	617
18,6	0,02	0,01	< 0,01		< 0,01		18	5	172	14	5,8E+03	818
19,6	0,03	0,01	< 0,01		< 0,01		< 1		247	20	2,6E+04	1,7E+03
20,6	0,08	0,02	< 0,01		< 0,01		5	2	46	7	4,1E+04	7,2E+03
21,7	< 0,01		< 0,01		< 0,01		< 1		< 1		50	25

Table A 4: Raw data of taken from the spigot of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa* WAM	± 95 % CI	*E. coli* WAM	± 95 % CI	*E. faecalis* WAM	± 95 % CI	CC 20°C WAM	± 95 % CI	CC 36°C WAM	± 95 % CI	HPC WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,6	n. d.		n. d.		n. d.		n. d.		n. d.		n. d.	
4,6	n. d.		n. d.		n. d.		n. d.		n. d.		n. d.	
5,6	< 0,01		< 0,01		< 0,01		n. d.		n. d.		n. d.	
6,6	< 0,01		< 0,01		< 0,01		n. d.		n. d.		n. d.	
7,6	< 0,01		< 0,01		< 0,01		17	4	21	5	933	346
8,6	< 0,01		< 0,01		< 0,01		< 1		2	3	542	79
9,6	< 0,01		< 0,01		< 0,01		45	7	47	7	228	52
10,6	< 0,01		< 0,01		< 0,01		6	5	10	4	491	76
11,6	< 0,01		< 0,01		< 0,01		8	5	16	5	664	88
12,6	< 0,01		< 0,01		< 0,01		44	9	18	5	503	77
13,6	< 0,01		< 0,01		< 0,01		9	3	5	3	485	75
14,6	< 0,01		< 0,01		< 0,01		9	6	1	2	524	78
15,6	< 0,01		< 0,01		< 0,01		7	3	7	3	791	115
16,6	< 0,01		< 0,01		< 0,01		7	3	16	4	1,9E+03	149
17,6	< 0,01		< 0,01		< 0,01		< 1		< 1		575	83
18,6	< 0,01		< 0,01		< 0,01		20	5	5	2	64	27
19,6	< 0,01		< 0,01		< 0,01		8	3	6	3	1,7E+03	140
20,6	< 0,01		< 0,01		< 0,01		11	4	31	6	1,8E+03	146
21,7	< 0,01		< 0,01		< 0,01		< 1		< 1		91	33
24,6	< 0,01		< 0,01		< 0,01		12	4	75	10	536	79

Biofilm samples – Rig Ref

Table A 5: Raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	63	35	110	27	1,1E+03	189	3,4E+05	8,0E+04
4,6	< 0,06	< 0,06	< 0,06	4	0	35	20	3,2E+03	221	4,5E+05	1,2E+05
8,6	< 0,06	< 0,06	< 0,06	2	2	217	16	8,1E+03	844	4,7E+05	1,3E+05
12,6	< 0,06	< 0,06	< 0,06	2	2	40	5	1,0E+04	862	1,3E+06	1,9E+05
16,6	< 0,06	< 0,06	< 0,06	2	1	644	68	9,5E+03	1,2E+03	2,3E+05	4,8E+04
20,6	< 0,06	< 0,06	< 0,06	10	3	21	4	7,9E+03	705	2,3E+05	4,9E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		1	1	19	12	4,7E+05	1,1E+05

Table A 6: Raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	50	9	64	9	416	36	3,0E+05	1,2E+05
4,6	< 0,06	< 0,06	< 0,06	61	4	463	869	3,1E+03	1,7E+03	3,0E+05	8,4E+04
8,6	< 0,06	< 0,06	< 0,06	1	1	42	6	8,2E+03	834	1,8E+05	5,8E+04
12,6	< 0,06	< 0,06	< 0,06	6	2	108	13	1,4E+04	942	1,2E+06	4,7E+05
16,6	< 0,06	< 0,06	< 0,06	3	1	114	10	1,0E+04	809	1,5E+05	3,5E+04
20,6	< 0,06	< 0,06	< 0,06	21	4	169	10	1,1E+04	860	1,6E+05	2,1E+04
21,7	< 0,06	< 0,06	< 0,06	1	1	16	3	45	17	2,9E+04	1,5E+04

Table A 7: Raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	31	11	38	8	1,6E+03	332	5,3E+03	4,6E+03
4,6	< 0,06	< 0,06	< 0,06	4	1	37	19	5,2E+03	1,8E+03	3,4E+05	5,9E+04
8,6	< 0,06	< 0,06	< 0,06	< 0,6		15	4	3,2E+03	418	2,8E+04	9,2E+03
12,6	< 0,06	< 0,06	< 0,06	3	2	4	1	5,7E+03	470	7,6E+05	2,0E+05
16,6	< 0,06	< 0,06	< 0,06	1	1	124	12	7,7E+03	578	8,0E+04	1,4E+04
20,6	< 0,06	< 0,06	< 0,06	1	1	15	2	1,7E+04	2,8E+03	9,8E+04	2,3E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		2	1	28	11	2,3E+05	5,9E+04

Table A 8: Raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	< 0,6		35	1	667	106	7,1E+04	2,8E+04
4,6	< 0,06	< 0,06	< 0,06	1	1	13	4	358	21	1,7E+05	4,5E+04
8,6	< 0,06	< 0,06	< 0,06	7	2	4	2	1,4E+03	271	7,1E+04	1,5E+04
12,6	< 0,06	< 0,06	< 0,06	1	1	12	2	4,2E+03	353	3,8E+04	8,9E+03
16,6	< 0,06	< 0,06	< 0,06	1	1	3	1	3,2E+03	357	3,1E+04	6,8E+03
20,6	< 0,06	< 0,06	< 0,06	5	1	3	1	3,2E+03	2,4E+03	7,1E+04	1,1E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		3	1	27	13	2,1E+04	2,5E+03

Biofilm samples – Rig Con

Table A 9: Raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	12	4	33	25	3,5E+04	1,2E+03	4,0E+04	1,8E+04
4,6	< 0,06	< 0,06	< 0,06	5	2	87	20	1,0E+06	1,1E+05	8,8E+05	2,1E+05
8,6	25,23	< 0,06	< 0,06	7	2	33	5	7,9E+03	822	1,4E+05	3,5E+04
12,6	< 0,06	< 0,06	< 0,06	12	3	42	5	9,0E+03	773	1,3E+06	3,6E+05
16,6	< 0,06	< 0,06	< 0,06	1	1	72	7	9,5E+03	786	1,3E+05	2,5E+04
20,6	< 0,06	< 0,06	< 0,06	5	2	26	4	3,4E+04	4,9E+03	2,3E+05	2,8E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		4	2	198	36	2,1E+05	4,0E+04

Table A 10: Raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	3	2	74	20	5,9E+03	1,0E+03	2,7E+05	7,9E+04
4,6	< 0,06	< 0,06	< 0,06	13	2	455	60	4,1E+04	1,1E+03	7,7E+05	2,1E+05
8,6	54,00	< 0,06	< 0,06	49	6	2,3E+04	1,5E+03	8,0E+03	818	2,9E+05	3,8E+04
12,6	59,64	< 0,06	< 0,06	7	2	2,3E+03	378	1,3E+04	914	4,8E+06	3,7E+06
16,6	3,87	< 0,06	< 0,06	1	1	279	44	1,0E+04	806	1,4E+05	2,7E+04
20,6	< 0,06	< 0,06	< 0,06	16	3	71	7	7,3E+03	689	2,7E+05	6,7E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		3	2	40	17	1,6E+06	3,2E+05

Table A 11: Raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	9	2	16	6	1,4E+04	4,1E+03	3,1E+05	7,8E+04
4,6	< 0,06	< 0,06	< 0,06	14	2	127	93	9,5E+04	2,2E+04	1,1E+05	2,8E+04
8,6	1,00	< 0,06	< 0,06	7	2	30	4	3,1E+03	398	2,0E+05	5,4E+04
12,6	< 0,06	< 0,06	< 0,06	12	2	34	4	5,7E+03	470	8,8E+05	3,5E+05
16,6	< 0,06	< 0,06	< 0,06	7	7	14	2	7,2E+03	538	1,1E+05	1,5E+04
20,6	< 0,06	< 0,06	< 0,06	3	1	18	3	4,4E+03	414	1,6E+05	2,4E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		3	1	37	13	1,1E+06	1,2E+05

Table A 12: Raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 1)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,6	< 0,06	< 0,06	< 0,06	1	0	12	6	1,6E+03	574	1,0E+05	2,8E+04
4,6	< 0,06	< 0,06	< 0,06	2	4	31	9	1,0E+03	262	3,0E+04	4,5E+04
8,6	< 0,06	< 0,06	< 0,06	14	3	15	3	1,5E+03	279	8,1E+04	1,2E+04
12,6	< 0,06	< 0,06	< 0,06	11	2	52	5	6,5E+03	548	5,9E+05	1,3E+05
16,6	< 0,06	< 0,06	< 0,06	7	10	37	4	3,3E+03	367	8,3E+04	1,4E+04
20,6	< 0,06	< 0,06	< 0,06	1	1	6	2	1,3E+04	2,4E+03	9,4E+04	1,3E+04
21,7	< 0,06	< 0,06	< 0,06	< 0,6		1	1	37	13	8,7E+04	8,5E+03

10.2 Raw data of TS 2

Water samples – Rig Ref

Table A 13: Raw data of tank water taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 2)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,9E+04	1,5E+03
2,0	< 0,01		< 0,01		< 0,01		3	2	88	11	2,8E+03	289
3,0	< 0,01		< 0,01		< 0,01		4	4	57	8	6,6E+04	1,1E+04
4,0	< 0,01		< 0,01		< 0,01		3	2	221	16	2,9E+03	183
5,0	< 0,01		< 0,01		< 0,01		2	2	49	8	2,3E+03	513
8,1	< 0,01		< 0,01		< 0,01		2	2	1	2	265	55

Water samples – Rig Con

Table A 14: Raw data of tank water taken from Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,1E+05	1,2E+04
2,0	< 0,01		< 0,01		< 0,01		7	4	> 300		3,9E+04	2,2E+03
4,0	< 0,01		< 0,01		< 0,01		4	2	643	86	6,9E+04	9,1E+03
5,0	< 0,01		< 0,01		< 0,01		11	4	306	61	5,0E+04	7,6E+03
8,1	< 0,01		< 0,01		< 0,01		< 1		4	2	124	38

Table A 15: Raw data of taken from the faucet of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		< 1		< 1		4,8E+04	7,9E+03
2,0	0,17		< 0,01		< 0,01		11	4	> 300		2,1E+04	1,7E+03
4,0	0,06		< 0,01		< 0,01		5	4	399	68	3,0E+04	1,8E+03
5,0	< 0,01		< 0,01		< 0,01		5	3	337	62	2,5E+04	1,7E+03
8,1	< 0,01		< 0,01		< 0,01		< 1		5	3	80	32

Table A 16: Raw data of taken from the spigot of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		< 1		< 1		90	34
2,0	< 0,01		< 0,01		< 0,01		12	4	75	10	536	79
4,0	< 0,01		< 0,01		< 0,01		6	3	26	6	755	95
5,0	< 0,01		< 0,01		< 0,01		5	2	29	6	715	91
8,1	< 0,01		< 0,01		< 0,01		< 1		5	3	72	29

Biofilm samples – Rig Ref

Table A 17: Raw data of biofilm section Bs 1 of Rig Ref at 36°C operating temperature and dosage of 0 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	51	6	401	51	7,4E+03	698	3,1E+05	4,9E+04
5,0	< 0,06	< 0,06	< 0,06	4	2	145	33	3,2E+03	494	8,9E+05	1,3E+05
8,1	< 0,06	< 0,06	< 0,06	6	11	< 0,6		31	15	1,1E+05	1,9E+04

Table A 18: Raw data of biofilm section Bs 2 of Rig Ref at 36°C operating temperature and dosage of 0 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	41	5	145	10	1,2E+03	87	2,9E+05	5,3E+04
5,0	< 0,06	< 0,06	< 0,06	3	1	258	40	4,5E+03	527	7,1E+05	1,5E+05
8,1	< 0,06	< 0,06	< 0,06	< 0,6		1	1	25	15	8,2E+04	9,5E+03

Table A 19: Raw data of biofilm section Bs 3 of Rig Ref at 36°C operating temperature and dosage of 0 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	26	3	209	29	6,8E+03	520	3,5E+05	6,4E+04
5,0	< 0,06	< 0,06	< 0,06	1	1	94	20	3,7E+03	390	2,9E+05	5,0E+04
8,1	< 0,06	< 0,06	< 0,06	3	1	2	1	56	16	4,0E+04	1,0E+04

Table A 20: Raw data of biofilm section Bs 4 of Rig Ref at 36°C operating temperature and dosage of 0 µg/L Na-acetate (TS 2)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	7	2	161	27	2,5E+03	397	1,7E+05	4,6E+04
5,0	< 0,06	< 0,06	< 0,06	1	1	44	4	5,2E+03	557	2,9E+05	7,9E+04
8,1	< 0,06	< 0,06	< 0,06	1	1	3	1	38	9	3,8E+04	8,0E+03

Biofilm samples – Rig Con

Table A 21: Raw data of biofilm section Bs 1 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	22	4	4,6E+03	577	1,3E+04	908	9,3E+04	3,2E+04
5,0	< 0,06	< 0,06	< 0,06	6	2	1,3E+04	889	7,0E+04	6,6E+03	4,7E+05	6,6E+04
8,1	< 0,06	< 0,06	< 0,06	< 0,6		2	1	86	41	3,4E+04	9,0E+03

Table A 22: Raw data of biofilm section Bs 2 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	4,77	< 0,06	< 0,06	176	17	1,0E+04	863	1,1E+04	858	6,1E+05	1,5E+05
5,0	< 0,06	< 0,06	< 0,06	6	2	3,7E+03	481	4,8E+04	5,5E+03	4,5E+05	9,6E+04
8,1	< 0,06	< 0,06	< 0,06	1	1	< 0,6		16	13	2,3E+04	6,8E+03

Table A 23: Raw data of biofilm section Bs 3 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	9	2	1,4E+03	201	1,6E+04	2,1E+03	2,9E+05	6,6E+04
5,0	< 0,06	< 0,06	< 0,06	2	1	985	206	9,4E+03	632	2,0E+05	4,5E+04
8,1	< 0,06	< 0,06	< 0,06	1	1	2	1	23	10	2,3E+04	8,6E+03

Table A 24: Raw data of biofilm section Bs 4 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 2)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	16	3	934	74	3,9E+03	397	4,1E+04	5,9E+03
5,0	< 0,06	< 0,06	< 0,06	3	1	597	51	7,6E+03	581	1,6E+05	3,9E+04
8,1	< 0,06	< 0,06	< 0,06	3	1	3	1	16	9	1,8E+04	6,9E+03

10.3 Raw data of TS 3

Water samples – Rig Ref

Table A 25: Raw data of tank water taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,0	< 0,01		< 0,01		< 0,01		< 1		68	9	2,5E+03	170
2,0	< 0,01		< 0,01		< 0,01		3	2	52	8	2,2E+03	158
3,0	< 0,01		< 0,01		< 0,01		2	3	125	12	4,8E+03	749
4,0	< 0,01		< 0,01		< 0,01		3	3	75	9	9,8E+03	1,1E+03
5,0	< 0,01		< 0,01		< 0,01		24	5	47	7	3,1E+03	299
6,0	< 0,01		< 0,01		< 0,01		n. d.		n. d.		7,2E+03	912
7,0	< 0,01		< 0,01		< 0,01		7	3	54	8	1,1E+04	1,1E+03
8,0	< 0,01		< 0,01		< 0,01		11	5	317	64	1,0E+04	1,1E+03
9,0	< 0,01		< 0,01		< 0,01		5	3	14	4	1,1E+04	1,1E+03
10,0	< 0,01		< 0,01		< 0,01		2	2	148	42	9,6E+03	1,1E+03
11,0	< 0,01		< 0,01		< 0,01		2	3	126	12	7,0E+03	901
12,0	< 0,01		< 0,01		< 0,01		< 1		7	3	4,3E+03	708
13,0	< 0,01		< 0,01		< 0,01		< 1		7	3	6,8E+03	904
14,0	< 0,01		< 0,01		< 0,01		< 1		37	7	2,1E+03	154
15,0	< 0,01		< 0,01		< 0,01		6	3	25	5	1,9E+03	149
16,0	< 0,01		< 0,01		< 0,01		1	2	55	8	2,4E+03	167
17,0	< 0,01		< 0,01		< 0,01		2	2	30	6	2,3E+03	163
18,0	< 0,01		< 0,01		< 0,01		10	20	6	3	1,7E+03	141
19,0	< 0,01		< 0,01		< 0,01		< 1		5	2	1,0E+03	108
20,0	< 0,01		< 0,01		< 0,01		< 1		3	2	1,4E+03	128
21,0	< 0,01		< 0,01		< 0,01		< 1		4	2	1,6E+03	138
22,0	< 0,01		< 0,01		< 0,01		3	3	2	2	1,0E+03	109
22,1	< 0,01		< 0,01		< 0,01		< 1		< 1		< 1	

Water samples – Rig Con

Table A 26: Raw data of tank water taken from Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
1,0	< 0,01		< 0,01		< 0,01		1	2	224	16	1,4E+05	1,4E+04
2,0	< 0,01		< 0,01		< 0,01		1	1	155	13	1,3E+05	1,2E+04
3,0	< 0,01		< 0,01		< 0,01		3	3	165	14	1,3E+05	1,2E+04
4,0	< 0,01		< 0,01		< 0,01		< 1		118	12	1,7E+05	1,4E+04
5,0	< 0,01		< 0,01		< 0,01		17	4	94	10	1,4E+05	1,3E+04
6,0	< 0,01		< 0,01		< 0,01		n. d.		n. d.		6,2E+04	8,5E+03
7,0	< 0,01		< 0,01		< 0,01		5	4	52	8	5,8E+04	8,2E+03
8,0	< 0,01		< 0,01		< 0,01		10	3	439	72	7,5E+04	1,1E+04
9,0	< 0,01		< 0,01		< 0,01		< 1		128	12	1,7E+05	1,4E+04
10,0	< 0,01		< 0,01		< 0,01		10	3	152	42	1,4E+05	1,3E+04
11,0	< 0,01		< 0,01		< 0,01		3	2	124	38	1,2E+05	1,2E+04
12,0	< 0,01		< 0,01		< 0,01		< 1		68	9	9,0E+04	1,0E+04
13,0	0,01	3,6E-03	< 0,01		< 0,01		< 1		284	29	9,4E+04	1,0E+04
14,0	0,03	6,2E-03	< 0,01		< 0,01		< 1		172	14	4,9E+04	7,7E+03
15,0	< 0,01		< 0,01		0,01	6,2E-03	5	4	106	11	5,2E+04	7,8E+03
16,0	< 0,01		< 0,01		< 0,01		5	4	485	75	3,8E+04	3,3E+03
17,0	< 0,01		< 0,01		< 0,01		7	3	31	10	6,1E+04	8,4E+03
18,0	< 0,01		< 0,01		< 0,01		< 1		64	9	7,9E+04	9,6E+03
19,0	< 0,01		< 0,01		< 0,01		< 1		10	3	6,8E+04	9,3E+03
20,0	< 0,01		< 0,01		< 0,01		< 1		13	4	2,6E+04	2,1E+03
21,0	< 0,01		< 0,01		< 0,01		< 1		16	4	7,7E+04	1,6E+04
22,0	0,01	4,4E-03	< 0,01		< 0,01		3	3	18	5	6,5E+04	8,7E+03
22,1	< 0,01		< 0,01		< 0,01		< 1		< 1		< 1	

Table A 27: Raw data of taken from the faucet of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		1	2	< 10	
1,0	< 0,01		< 0,01		< 0,01		1	1	156	16	9,9E+04	1,1E+04
2,0	< 0,01		< 0,01		< 0,01		5	4	89	10	7,7E+04	9,5E+03
3,0	< 0,01		< 0,01		< 0,01		19	5	145	13	9,9E+04	1,1E+04
4,0	< 0,01		< 0,01		< 0,01		6		112	11	9,1E+04	1,0E+04
5,0	< 0,01		< 0,01		< 0,01		24	5	78	10	2,8E+04	2,9E+03
6,0	< 0,01		< 0,01		< 0,01		n. d.		n. d.		4,6E+04	7,3E+03
7,0	410,00	125,50	< 0,01		< 0,01		510	81	488	75	2,4E+04	1,7E+03
8,0	194,55	26,07	< 0,01		< 0,01		190	15	172	14	3,2E+04	3,1E+03
9,0	37,05	6,55	< 0,01		< 0,01		88		151	13	8,5E+04	9,9E+03
10,0	237,00	67,47	< 0,01		1,53	0,24	285	22	278	21	7,9E+04	9,6E+03
11,0	310,00	109,13	< 0,01		4,10	1,26	335	31	645	87	7,8E+04	9,5E+03
12,0	45,00	13,15	< 0,01		< 0,01		< 1		77	9	3,3E+04	6,3E+03
13,0	120,91	20,55	< 0,01		< 0,01		< 1		230	16	2,1E+04	1,8E+03
14,0	4,20	1,30	< 0,01		0,16	0,08	< 1		96	11	1,8E+04	1,5E+03
15,0	480,00	135,79	< 0,01		< 0,01		357	68	527	78	1,7E+04	1,4E+03
16,0	45,00	13,15	< 0,01		< 0,01		45	7	218	16	1,8E+04	1,7E+03
17,0	102,00	19,80	< 0,01		0,01	0,01	32	6	172	14	2,9E+04	5,8E+03
18,0	480,00	135,79	< 0,01		4,60	1,33	258		238	17	7,2E+04	9,1E+03
19,0	49,00	13,72	< 0,01		0,27	0,10	< 1		80	10	3,1E+04	3,0E+03
20,0	550,00	145,36	< 0,01		0,21	0,09	< 1		644	88	1,2E+04	1,2E+03
21,0	46,00	13,29	< 0,01		7,00	1,64	< 1		300	339	2,9E+04	7,1E+03
22,0	550,00	145,36	< 0,01		0,96	0,19	570	85	615	85	1,4E+04	1,3E+03
22,1	< 0,01		< 0,01		< 0,01		< 1		< 1		< 1	

Table A 28: Raw data of taken from the spigot of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		5	3	68	9	569	81
2,0	< 0,01		< 0,01		< 0,01		33	6	118	12	577	82
3,0	< 0,01		< 0,01		< 0,01		59	8	74	9	650	106
4,0	< 0,01		< 0,01		< 0,01		19		78	10	885	103
5,0	< 0,01		< 0,01		< 0,01		25	5	36	6	414	83
6,0	< 0,01		< 0,01		< 0,01		n. d.		n. d.		367	65
7,0	< 0,01		< 0,01		< 0,01		34	6	225	16	2,1E+03	155
8,0	< 0,01		< 0,01		< 0,01		35	6	51	8	895	102
9,0	< 0,01		< 0,01		< 0,01		4		49	8	5,5E+03	799
10,0	< 0,01		< 0,01		< 0,01		4	3	42	7	1,2E+03	116
11,0	< 0,01		< 0,01		< 0,01		1	1	24	5	910	103
12,0	< 0,01		< 0,01		< 0,01		< 1		20	5	921	103
13,0	< 0,01		< 0,01		< 0,01		< 1		121	12	2,1E+03	156
14,0	< 0,01		< 0,01		< 0,01		< 1		17	4	416	71
15,0	< 0,01		< 0,01		< 0,01		13	4	148	13	6,3E+03	859
16,0	< 0,01		< 0,01		< 0,01		4	2	64	9	1,1E+03	112
17,0	< 0,01		< 0,01		< 0,01		7	3	15	4	410	69
18,0	< 0,01		< 0,01		< 0,01		16		11	4	419	70
19,0	< 0,01		< 0,01		< 0,01		< 1		101	11	1,2E+03	119
20,0	< 0,01		< 0,01		< 0,01		< 1		7	3	297	59
21,0	< 0,01		< 0,01		< 0,01		< 1		8	3	1,4E+03	152
22,0	< 0,01		< 0,01		< 0,01		5	3	9	3	318	75
22,1	< 0,01		< 0,01		< 0,01		< 1		< 1		< 1	

Biofilm samples – Rig Ref

Table A 29: Raw data of biofilm section Bs 1 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	2	1	70	7	767	69	4,4E+04	2,2E+04
5,0	< 0,06	< 0,06	< 0,06	8	2	12	3	5,5E+03	707	8,4E+04	2,6E+04
10,0	< 0,06	< 0,06	< 0,06	8	2	43	17	2,1E+04	6,5E+03	1,6E+05	2,8E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	1,4E+04	938	3,8E+05	1,8E+05
18,0	< 0,06	< 0,06	< 0,06	1	1	18	3	1,3E+04	892	3,7E+05	6,1E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	< 0,6		8,4E+03	750	3,8E+05	6,3E+04
22,1	< 0,06	< 0,06	< 0,06	1	1	10	3	26	17	1,2E+04	6,4E+03

Table A 30: FISH raw data of biofilm section Bs 1 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 31: FISH raw data of biofilm section Bs 2 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	16	3	39	5	971	78	4,3E+04	9,3E+03
5,0	< 0,06	< 0,06	< 0,06	4	2	5	2	2,4E+03	386	2,4E+05	5,4E+04
10,0	< 0,06	< 0,06	< 0,06	5	2	61	7	4,0E+03	502	6,5E+04	2,8E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		5	2	2,8E+03	420	3,6E+05	9,0E+04
18,0	< 0,06	< 0,06	< 0,06	1	1	3	2	1,7E+03	103	3,8E+05	7,6E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	6	2	2,9E+03	438	2,5E+05	4,5E+04
22,1	< 0,06	< 0,06	< 0,06	1	2	12	3	20	15	7,9E+03	2,6E+03

Table A 32: FISH raw data of biofilm section Bs 2 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 33: Raw data of biofilm section Bs 3 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	2	1	21	3	1,5E+03	251	3,1E+04	3,9E+03
5,0	< 0,06	< 0,06	< 0,06	5	1	12	2	2,2E+03	308	1,6E+05	6,7E+04
10,0	< 0,06	< 0,06	< 0,06	1	1	7	2	5,2E+03	533	3,6E+05	1,0E+05
14,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	3,5E+03	402	1,2E+05	5,3E+04
18,0	< 0,06	< 0,06	< 0,06	5	1	10	2	5,0E+03	454	2,2E+05	4,5E+04
22,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	1,5E+04	2,6E+03	1,5E+05	3,1E+04
22,1	< 0,06	< 0,06	< 0,06	1	1	1	1	< 6		7,9E+03	3,4E+03

Table A 34: FISH raw data of biofilm section Bs 3 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 35: FISH raw data of biofilm section Bs 4 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		446	43	1,2E+03	446	1,3E+04	3,0E+03
5,0	< 0,06	< 0,06	< 0,06	22	3	36	4	456	536	2,5E+05	5,5E+04
10,0	< 0,06	< 0,06	< 0,06	2	1	11	2	893	3,1E+03	3,9E+04	1,2E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	1,2E+03	1,1E+03	2,0E+05	3,9E+04
18,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	5,7E+03	1,1E+03	2,6E+05	7,1E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	2	1	5,4E+03	473	1,5E+05	2,9E+04
22,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		7	10	1,6E+04	8,2E+03

Table A 36: FISH raw data of biofilm section Bs 4 taken from Rig Ref at 36°C operating temperature and without dosage of Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Biofilm samples – Rig Con

Table A 37: Raw data of biofilm section Bs 1 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	12	3	24	13	1,7E+04	3,3E+03	4,6E+04	9,7E+03
5,0	< 0,06	< 0,06	< 0,06	2	2	135	29	1,4E+05	9,3E+03	7,1E+05	4,3E+05
10,0	< 0,06	< 0,06	0,16	2	1	1,8E+03	169	7,1E+04	6,7E+03	3,8E+05	1,4E+05
14,0	0,04	< 0,06	< 0,06	< 0,6		40	15	2,4E+05	3,4E+04	2,3E+05	3,7E+04
18,0	< 0,06	< 0,06	< 0,06	2	1	8	2	1,8E+05	1,7E+04	3,3E+05	2,9E+05
22,0	< 0,06	< 0,06	< 0,06	1	1	2	1	1,3E+05	9,2E+03	5,6E+05	1,0E+05
22,1	< 0,06	< 0,06	< 0,06	1	1	2	1	11	11	1,4E+04	7,5E+03

Table A 38: FISH raw data of biofilm section Bs 1 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 39: Raw data of biofilm section Bs 2 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	6	2	60	20	8,0E+03	711	3,3E+04	5,2E+03
5,0	< 0,06	< 0,06	< 0,06	1	1	63	20	5,8E+04	6,1E+03	3,4E+05	6,6E+04
10,0	< 0,06	< 0,06	0,16	2	1	75	22	1,2E+05	8,6E+03	4,1E+05	7,9E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		59	21	2,8E+04	4,4E+03	1,8E+05	2,5E+04
18,0	< 0,06	< 0,06	< 0,06	9	2	26	4	1,7E+04	1,2E+03	2,2E+05	4,5E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	10	3	3,5E+04	5,9E+03	2,5E+05	8,1E+04
22,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		5,4E+03	2,3E+03

Table A 40: FISH raw data of biofilm section Bs 2 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	+	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 41: Raw data of biofilm section Bs 3 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	1	1	121	22	3,9E+03	483	1,5E+04	4,5E+03
5,0	< 0,06	< 0,06	< 0,06	10	2	97	20	2,5E+04	3,2E+03	2,1E+05	5,6E+04
10,0	< 0,06	< 0,06	< 0,06	7	2	59	16	3,6E+04	3,9E+03	2,4E+05	4,1E+04
14,0	0,04	< 0,06	< 0,06	< 0,6		46	14	1,7E+04	2,7E+03	2,1E+05	2,6E+04
18,0	< 0,06	< 0,06	< 0,06	1	1	2	1	2,0E+04	2,8E+03	1,4E+05	3,5E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	1	1	2,6E+04	3,9E+03	1,5E+05	3,4E+04
22,1	< 0,06	< 0,06	< 0,06	1	1	1	1	< 0,6		6,6E+03	8,8E+03

Table A 42: FISH raw data of biofilm section Bs 3 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 43: Raw data of biofilm section Bs 4 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
2,0	< 0,06	< 0,06	< 0,06	2	1	1,1E+03	108	4,9E+03	450	1,8E+04	4,6E+03
5,0	< 0,06	< 0,06	< 0,06	5	1	109	22	7,1E+03	541	1,6E+05	2,9E+04
10,0	< 0,06	< 0,06	< 0,06	2	1	70	17	2,6E+04	3,3E+03	5,4E+04	1,0E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		14	8	1,1E+04	1,1E+03	6,0E+04	1,3E+04
18,0	< 0,06	< 0,06	< 0,06	1	1	3	1	1,2E+04	1,1E+03	1,4E+05	1,6E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	15	3	4,3E+03	415	2,2E+05	5,2E+04
22,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		3,8E+03	1,2E+03

Table A 44: FISH raw data of biofilm section Bs 4 of Rig Con at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 3)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

10.4 Raw data of TS 4

Water samples – Rig Ref

Table A 45: Raw data of tank water taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 4)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
2,0	< 0,01		< 0,01		< 0,01		< 1		6	3	934	104
3,0	< 0,01		< 0,01		< 0,01		1	2	1	2	774	95
4,0	< 0,01		< 0,01		< 0,01		< 1		1	2	688	89
5,0	< 0,01		< 0,01		< 0,01		< 1		1	2	396	68

Water samples – Rig Con

Table A 46: Raw data of tank water taken from Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
2,0	< 0,01		< 0,01		< 0,01		< 1		5	3	1,2E+04	1,2E+03
3,0	< 0,01		< 0,01		< 0,01		< 1		37	7	3,2E+03	306
4,0	< 0,01		< 0,01		< 0,01		< 1		140	13	5,2E+04	7,7E+03
5,0	< 0,01		< 0,01		< 0,01		< 1		65	9	1,2E+03	371
6,0	< 0,01		< 0,01		< 0,01		< 1		34	7	1,7E+03	453
7,0	< 0,01		< 0,01		< 0,01		< 1		31	6	1,3E+04	1,2E+03
8,0	< 0,01		< 0,01		< 0,01		< 1		41	7	1,7E+03	142
9,0	< 0,01		< 0,01		< 0,01		< 1		19	5	1,3E+03	123

Table A 47: Raw data of taken from the faucet of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
2,0	< 0,01		< 0,01		< 0,01		< 1		95	11	1,3E+04	1,2E+03
3,0	< 0,01		< 0,01		< 0,01		< 1		68	9	9,6E+03	1,1E+03
4,0	0,02		< 0,01		< 0,01		< 1		75	9	1,3E+04	1,2E+03
5,0	< 0,01		< 0,01		< 0,01		< 1		45	8	1,6E+03	433
6,0	0,01		< 0,01		< 0,01		3	2	16	4	1,2E+03	366
7,0	0,01		< 0,01		< 0,01		1	2	25	6	1,4E+04	1,3E+03
8,0	< 0,01		< 0,01		< 0,01		< 1		38	7	1,2E+03	120
9,0	< 0,01		< 0,01		< 0,01		3	3	13	4	883	101

Table A 48: Raw data of taken from the spigot of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa* WAM cfu/mL	± 95 % CI cfu/mL	*E. coli* WAM cfu/mL	± 95 % CI cfu/mL	*E. faecalis* WAM cfu/mL	± 95 % CI cfu/mL	CC 20°C WAM cfu/mL	± 95 % CI cfu/mL	CC 36°C WAM cfu/mL	± 95 % CI cfu/mL	HPC WAM cfu/mL	± 95 % CI cfu/mL
2,0	< 0,01		< 0,01		< 0,01		1	1	22	5	238	52
3,0	< 0,01		< 0,01		< 0,01		< 1		55	8	208	49
4,0	< 0,01		< 0,01		< 0,01		10	20	217	16	4,4E+03	718
5,0	< 0,01		< 0,01		< 0,01		< 1		5	3	293	58
6,0	< 0,01		< 0,01		< 0,01		< 1		13	4	289	58
7,0	< 0,01		< 0,01		< 0,01		< 1		4	2	338	64
8,0	< 0,01		< 0,01		< 0,01		< 1		< 1		139	40
9,0	< 0,01		< 0,01		< 0,01		2	3	7	3	370	66

Biofilm samples – Rig Ref

Table A 49: Raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	< 0,6		3	2	648	66	8,5E+04	2,1E+04

Table A 50: Raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	< 0,6		3	3	70	22	1,8E+05	4,9E+04

Table A 51: Raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	1	1	30	3	918	93	5,1E+04	1,8E+04

Table A 52: Raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	< 0,6		47	5	1,8E+03	684	1,6E+05	4,2E+04

Biofilm samples – Rig Con

Table A 53: Raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	< 0,6		39	21	6,5E+04	6,5E+03	8,4E+05	2,5E+05
6,0	< 0,06	< 0,06	< 0,06	2	2	89	25	1,4E+04	3,1E+03	3,6E+05	6,1E+04

Table A 54: Raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	1	1	667	67	9,3E+04	7,9E+03	5,8E+05	1,1E+05
6,0	< 0,06	< 0,06	< 0,06	3	2	90	25	1,1E+04	2,8E+03	4,9E+05	6,7E+04

Table A 55: Raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	2	1	23	11	1,8E+04	2,5E+03	2,7E+05	2,9E+04
6,0	< 0,06	< 0,06	< 0,06	2	1	13	7	6,2E+03	1,5E+03	2,2E+05	4,8E+04

Table A 56: Raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 4)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
3,0	< 0,06	< 0,06	< 0,06	29	3	1,0E+03	103	4,6E+03	691	5,0E+04	1,3E+04
6,0	< 0,06	< 0,06	< 0,06	1	1	82	23	3,6E+03	382	1,4E+05	2,0E+04

10.5 Raw data of TS 5

Water samples – Rig Ref

Table A 57: Raw data of tank water taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		2	2	3	2	526	79
1,9	< 0,01		< 0,01		< 0,01		< 1		6	3	742	93
3,0	< 0,01		< 0,01		< 0,01		< 1		5	2	1,1E+03	110
4,0	< 0,01		< 0,01		< 0,01		< 1		2	2	937	104
4,9	< 0,01		< 0,01		< 0,01		5	2	5	3	844	99
6,0	< 0,01		< 0,01		< 0,01		< 1		4	2	1,6E+03	134
7,0	< 0,01		< 0,01		< 0,01		< 1		4	3	819	97
8,0	< 0,01		< 0,01		< 0,01		1	1	3	2	1,4E+03	128
9,0	< 0,01		< 0,01		< 0,01		< 1		3	2	2,4E+03	165
10,0	< 0,01		< 0,01		< 0,01		< 1		5	3	1,0E+03	109
11,0	< 0,01		< 0,01		< 0,01		7	3	8	3	1,6E+03	135
12,0	< 0,01		< 0,01		< 0,01		< 1		2	3	1,3E+03	121
13,0	< 0,01		< 0,01		< 0,01		< 1		33	7	1,9E+03	148
14,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
15,0	< 0,01		< 0,01		< 0,01		1	2	1	1	1,1E+03	110
16,0	< 0,01		< 0,01		< 0,01		57	9	1	1	1,1E+03	137
17,0	< 0,01		< 0,01		< 0,01		16	4	17	5	1,2E+03	118
18,0	< 0,01		< 0,01		< 0,01		< 1		22	5	970	106
19,0	< 0,01		< 0,01		< 0,01		1	2	6	3	793	96
20,0	< 0,01		< 0,01		< 0,01		< 1		< 1		580	98
21,0	< 0,01		< 0,01		< 0,01		1	2	1	2	652	87
21,9	< 0,01		< 0,01		< 0,01		1	2	1	2	839	100
22,7	< 0,01		< 0,01		< 0,01		< 1		< 1		172	45

Water samples – Rig Con

Table A 58: Raw data of tank water taken from Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		< 1		2	3	3,3E+03	631
1,9	< 0,01		< 0,01		< 0,01		4	4	3	2	3,8E+03	679
3,0	< 0,01		< 0,01		< 0,01		1	2	< 1		3,1E+03	606
4,0	< 0,01		< 0,01		< 0,01		< 1		6	3	7,1E+03	907
4,9	< 0,01		< 0,01		< 0,01		2	2	3	2	1,2E+04	1,2E+03
6,0	< 0,01		< 0,01		< 0,01		< 1		1	1	1,5E+04	1,3E+03
7,0	< 0,01		< 0,01		< 0,01		< 1		1	1	2,7E+04	1,8E+03
8,0	< 0,01		< 0,01		< 0,01		7	3	12	5	1,3E+04	1,2E+03
9,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,2E+04	1,2E+03
10,0	< 0,01		< 0,01		< 0,01		< 1		3	3	5,7E+03	814
11,0	< 0,01		< 0,01		< 0,01		23	5	8	3	8,9E+03	1,0E+03
12,0	< 0,01		< 0,01		< 0,01		1	2	1	2	1,8E+03	143
13,0	< 0,01		< 0,01		< 0,01		1	2	6	3	9,5E+03	1,0E+03
14,0	< 0,01		< 0,01		< 0,01		< 1		< 1		7,8E+03	950
15,0	< 0,01		< 0,01		< 0,01		< 1		2	3	7,2E+03	914
16,0	< 0,01		< 0,01		< 0,01		< 1		2	3	3,3E+03	309
17,0	< 0,01		< 0,01		< 0,01		< 1		< 1		6,2E+03	848
18,0	< 0,01		< 0,01		< 0,01		< 1		< 1		3,7E+03	668
19,0	< 0,01		< 0,01		< 0,01		3	2	5	2	8,2E+03	974
20,0	< 0,01		< 0,01		< 0,01		< 1		< 1		9,1E+03	1,0E+03
21,0	< 0,01		< 0,01		< 0,01		< 1		< 1		6,6E+03	888
21,9	< 0,01		< 0,01		< 0,01		19	5	25	6	6,8E+03	889
22,7	< 0,01		< 0,01		< 0,01		< 1		< 1		87	33

Table A 59: Raw data of taken from the faucet of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		5	2	747	98	5,9E+03	832
1,9	< 0,01		< 0,01		< 0,01		6	3	256	28	1,5E+04	1,3E+03
3,0	< 0,01		< 0,01		< 0,01		2	1	14	4	9,4E+03	1,0E+03
4,0	< 0,01		< 0,01		< 0,01		< 1		17	5	9,3E+03	1,0E+03
4,9	< 0,01		< 0,01		< 0,01		13	4	43	7	1,4E+04	1,3E+03
6,0	< 0,01		< 0,01		< 0,01		< 1		5	2	1,4E+04	1,3E+03
7,0	0,07	0,03	< 0,01		0,06	0,03	< 1		29	23	2,4E+04	1,7E+03
8,0	0,35	0,08	< 0,01		1,70	0,22	27	6	240	53	1,8E+04	1,4E+03
9,0	0,20	0,08	< 0,01		0,57	0,13	8	4	10	14	1,3E+04	1,2E+03
10,0	0,02	0,02	< 0,01		2,12	0,16	6	3	50	44	6,7E+03	883
11,0	< 0,01		< 0,01		64,67	9,10	83	10	154	13	8,2E+03	980
12,0	0,04	0,03	< 0,01		< 0,01		1	2	15	4	2,2E+03	158
13,0	0,26	0,07	< 0,01		0,05	0,03	1	1	33	6	6,3E+03	855
14,0	1,03	0,13	< 0,01		11,36	1,15	8	3	73	9	7,2E+03	912
15,0	1,28	0,15	< 0,01		0,27	0,06	3	2	45	7	6,9E+03	913
16,0	0,02	0,02	< 0,01		0,01	0,02	1	2	4	2	5,4E+03	792
17,0	< 0,01		< 0,01		< 0,01		2	3	6	3	5,6E+03	822
18,0	0,02	0,03	< 0,01		0,02	0,02	< 1		3	3	4,7E+03	886
19,0	1,45	0,13	< 0,01		0,55	0,08	4	2	5	2	4,6E+03	732
20,0	0,51	0,08	< 0,01		< 0,01		< 1		6	3	5,3E+03	788
21,0	0,01	0,01	< 0,01		0,04	0,02	< 1		4	2	4,6E+03	735
21,9	0,46	0,07	< 0,01		17,00	1,41	13	4	22	5	4,7E+03	739
22,7	< 0,01		< 0,01		< 0,01		< 1		< 1		2,1E+03	157

Table A 60: Raw data of taken from the spigot of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
0,0	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		1	2	17	4	938	106
1,9	< 0,01		< 0,01		< 0,01		5	3	24	5	1,2E+03	120
3,0	< 0,01		< 0,01		< 0,01		1	1	17	5	1,2E+03	118
4,0	< 0,01		< 0,01		< 0,01		< 1		32	6	4,8E+03	747
4,9	< 0,01		< 0,01		< 0,01		11	4	23	5	1,2E+03	141
6,0	< 0,01		< 0,01		< 0,01		2	2	6	3	6,7E+03	898
7,0	< 0,01		< 0,01		< 0,01		< 1		21	5	1,3E+03	392
8,0	< 0,01		< 0,01		< 0,01		23	6	36	7	8,4E+03	989
9,0	< 0,01		< 0,01		< 0,01		2	2	14	4	7,6E+03	939
10,0	< 0,01		< 0,01		< 0,01		2	3	9	3	3,3E+03	307
11,0	< 0,01		< 0,01		< 0,01		10	14	21	5	6,4E+03	865
12,0	< 0,01		< 0,01		< 0,01		2	1	6	3	613	276
13,0	< 0,01		< 0,01		< 0,01		40	7	8	3	485	238
14,0	< 0,01		< 0,01		< 0,01		< 1		4	2	563	260
15,0	< 0,01		< 0,01		< 0,01		< 1		11	4	3,5E+03	634
16,0	< 0,01		< 0,01		< 0,01		< 1		4	2	267	185
17,0	< 0,01		< 0,01		< 0,01		< 1		8	3	182	252
18,0	< 0,01		< 0,01		< 0,01		< 1		6	3	697	285
19,0	< 0,01		< 0,01		< 0,01		< 1		7	3	613	276
20,0	< 0,01		< 0,01		< 0,01		< 1		< 1		3,6E+03	651
21,0	< 0,01		< 0,01		< 0,01		< 1		1	1	1,0E+03	346
21,9	< 0,01		< 0,01		< 0,01		1	1	3	2	1,5E+03	429
22,7	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	

Biofilm samples – Rig Ref

Table A 61: Raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	2	1	11	3	2,7E+03	417	2,9E+05	9,4E+04
4,9	< 0,06	< 0,06	< 0,06	5	2	14	3	1,6E+03	122	1,5E+05	3,0E+04
10,0	< 0,06	< 0,06	< 0,06	1	1	2	1	6,0E+03	626	6,5E+04	6,2E+03
14,0	< 0,06	< 0,06	< 0,06	< 0,6		4	2	1,3E+04	895	2,2E+04	7,6E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		7,8E+03	706	1,4E+05	2,7E+04
21,9	< 0,06	< 0,06	< 0,06	2	1	21	4	3,1E+03	455	8,8E+04	2,3E+04
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		20	15	790	854

Table A 62: FISH raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 63: Raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	2	2	2	1	627	64	2,2E+05	4,5E+04
4,9	< 0,06	< 0,06	< 0,06	9	2	4	2	1,5E+03	97	1,8E+05	4,4E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	4,7E+03	550	6,6E+04	1,3E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		3	1	9,6E+03	763	1,7E+04	7,8E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	3,7E+03	491	1,7E+05	3,7E+04
21,9	< 0,06	< 0,06	< 0,06	< 0,6		6	2	2,5E+03	412	1,4E+05	2,7E+04
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		195	435

Table A 64: FISH raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 65: Raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	0	1	3	1	822	58	1,7E+05	4,7E+04
4,9	< 0,06	< 0,06	< 0,06	9	2	12	2	2,0E+03	292	1,1E+05	3,2E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	4,4E+03	448	3,9E+04	1,5E+04
14,0	< 0,06	< 0,06	< 0,06	1	1	3	1	1,8E+03	274	1,5E+04	2,8E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		1,1E+03	210	8,5E+04	1,5E+04
21,9	< 0,06	< 0,06	< 0,06	1	1	13	2	1,9E+03	251	6,4E+04	1,3E+04
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		244	339

Table A 66: FISH raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 67: Raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	< 0,6		22	3	4,3E+03	337	1,5E+05	4,4E+04
4,9	< 0,06	< 0,06	< 0,06	1	1	6	2	1,5E+03	390	4,2E+04	1,0E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	210	412	3,6E+04	6,3E+03
14,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	568	276	7,6E+03	4,3E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	178	44	2,3E+04	5,7E+03
21,9	< 0,06	< 0,06	< 0,06	1	1	2	1	414	598	8,3E+04	2,6E+04
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		61	201

Table A 68: FISH raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and without dosage of Na-acetate (TS 5)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Biofilm samples – Rig Con

Table A 69: Raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	3	2	60	24	7,6E+04	7,0E+03	3,7E+05	4,4E+04
4,9	< 0,06	< 0,06	< 0,06	10	2	14	10	3,0E+04	5,3E+03	1,3E+05	4,3E+04
10,0	< 0,06	< 0,06	0,06	< 0,6		97	9	2,1E+05	3,9E+04	4,2E+05	7,6E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		83	8	1,4E+05	9,6E+03	2,0E+05	9,0E+04
18,0	< 0,06	< 0,06	< 0,06	1	2	5	2	2,0E+05	4,6E+04	3,2E+05	3,7E+04
21,9	< 0,06	< 0,06	< 0,06	1	1	73	8	3,9E+05	5,8E+04	9,1E+05	1,9E+05
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		6	11	582	996

Table A 70: FISH raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 71: Raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	18	4	16	11	8,8E+04	7,7E+03	5,4E+05	1,1E+05
4,9	< 0,06	< 0,06	< 0,06	6	2	48	17	6,1E+04	6,1E+03	9,0E+04	2,6E+04
10,0	< 0,06	< 0,06	0,06	< 0,6		149	10	1,5E+04	996	1,4E+05	2,7E+04
14,0	< 0,06	< 0,06	0,05	< 0,6		33	5	4,9E+04	5,6E+03	5,4E+04	2,0E+04
18,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	9,9E+03	793	2,0E+05	6,3E+04
21,9	< 0,06	< 0,06	< 0,06	1	2	6	2	3,6E+04	4,9E+03	1,1E+05	2,6E+04
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		1,3E+03	2,4E+03

Table A 72: FISH raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 73: Raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	1	1	94	20	2,0E+04	2,9E+03	3,0E+05	4,7E+04
4,9	< 0,06	< 0,06	< 0,06	13	2	76	6	2,8E+04	3,4E+03	8,9E+04	2,2E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		69	5	1,1E+04	1,1E+03	7,0E+04	1,1E+04
14,0	< 0,06	< 0,06	< 0,06	1	1	15	3	2,3E+04	3,3E+03	1,6E+04	7,5E+03
18,0	< 0,06	< 0,06	< 0,06	1	1	1	1	4,5E+03	438	1,3E+05	2,8E+04
21,9	< 0,06	< 0,06	< 0,06	1	1	4	1	8,6E+03	588	1,9E+04	7,3E+03
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6	< 0,6	202	325

Table A 74: FISH raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 75: Raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
1,9	< 0,06	< 0,06	< 0,06	1	1	91	6	2,7E+03	337	2,5E+04	7,2E+03
4,9	< 0,06	< 0,06	< 0,06	21	3	28	3	3,5E+03	390	4,4E+04	9,7E+03
10,0	< 0,06	< 0,06	< 0,06	< 0,6		4	1	3,9E+03	401	5,3E+04	1,8E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		4	1	2,0E+03	286	5,0E+03	3,8E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	467	44	2,7E+04	5,2E+03
21,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		5,8E+03	582	1,5E+04	3,2E+03
22,7	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		66	209

Table A 76: FISH raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 5)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

10.6 Raw data of TS 6

Water samples – Rig Ref

Table A 77: Raw data of tank water taken from Rig Ref at 20°C operating temperature and dosage of 100 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		172	45
1,0	< 0,01		< 0,01		< 0,01		3	3	62	8	3,1E+04	3,0E+03
2,0	< 0,01		< 0,01		< 0,01		< 1		< 1		9,5E+03	1,1E+03
3,0	< 0,01		< 0,01		< 0,01		< 1		< 1		7,3E+03	2,9E+03
4,0	< 0,01		< 0,01		< 0,01		< 1		3	2	1,1E+04	1,1E+03
5,0	< 0,01		< 0,01		< 0,01		< 1		4	2	8,4E+03	989
6,0	< 0,01		< 0,01		< 0,01		< 1		2	2	5,0E+03	758
7,0	< 0,01		< 0,01		< 0,01		< 1		< 1		4,9E+03	756
8,0	< 0,01		< 0,01		< 0,01		< 1		1	1	6,9E+03	893
9,0	< 0,01		< 0,01		< 0,01		1	2	< 1		1,0E+04	1,1E+03
11,0	< 0,01		< 0,01		< 0,01		3	3	1	2	8,1E+03	970
12,0	< 0,01		< 0,01		< 0,01		1	2	3	3	5,0E+03	761
13,0	< 0,01		< 0,01		< 0,01		< 1		< 1		4,2E+03	701
14,0	< 0,01		< 0,01		< 0,01		< 1		< 1		3,4E+03	624
15,0	< 0,01		< 0,01		< 0,01		< 1		< 1		2,5E+03	546
16,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,7E+03	141
16,8	< 0,01		< 0,01		< 0,01		< 1		< 1		2,2E+03	159
18,0	< 0,01		< 0,01		< 0,01		< 1		< 1		3,9E+03	213
19,0	< 0,01		< 0,01		< 0,01		< 1		< 1		4,2E+03	696
20,0	< 0,01		< 0,01		< 0,01		1	2	1	2	3,2E+03	606
21,0	< 0,01		< 0,01		< 0,01		< 1		< 1		3,9E+03	672
22,0	< 0,01		< 0,01		< 0,01		1	1	1	1	4,0E+03	690
22,8	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	

Water samples – Rig Con

Table A 78: Raw data of tank water taken from Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		87	33
1,0	< 0,01		< 0,01		< 0,01		17	4	47	7	2,8E+04	1,8E+03
2,0	< 0,01		< 0,01		< 0,01		< 1		2	2	1,3E+04	1,2E+03
3,0	< 0,01		< 0,01		< 0,01		1	2	< 1		6,2E+03	846
4,0	< 0,01		< 0,01		< 0,01		< 1		6	3	6,6E+03	873
5,0	< 0,01		< 0,01		< 0,01		< 1		4	2	2,0E+04	1,5E+03
6,0	< 0,01		< 0,01		< 0,01		< 1		2	2	1,9E+04	1,5E+03
7,0	< 0,01		< 0,01		< 0,01		< 1		1	2	2,2E+04	5,0E+03
8,0	< 0,01		< 0,01		< 0,01		< 1		5	3	1,2E+04	1,2E+03
9,0	< 0,01		< 0,01		< 0,01		< 1		2	3	1,2E+04	1,2E+03
11,0	< 0,01		< 0,01		< 0,01		< 1		< 1		4,5E+03	730
12,0	< 0,01		< 0,01		< 0,01		< 1		2	3	1,7E+04	1,4E+03
13,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,5E+04	1,3E+03
14,0	< 0,01		< 0,01		< 0,01		1	2	< 1		1,5E+04	1,3E+03
15,0	< 0,01		< 0,01		< 0,01		< 1		1	2	1,3E+04	1,2E+03
16,0	< 0,01		< 0,01		< 0,01		< 1		1	2	1,2E+04	1,2E+03
16,8	< 0,01		< 0,01		< 0,01		< 1		< 1		1,6E+04	1,4E+03
18,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,2E+04	1,2E+03
19,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,1E+04	3,6E+03
20,0	< 0,01		< 0,01		< 0,01		< 1		< 1		1,3E+04	1,2E+03
21,0	< 0,01		< 0,01		< 0,01		1	2	2	3	1,2E+04	1,2E+03
22,0	< 0,01		< 0,01		< 0,01		< 1		1	2	1,3E+04	1,3E+03
22,8	< 0,01		< 0,01		< 0,01		< 1		1	2	< 10	

Table A 79: Raw data of taken from the faucet of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		2,1E+03	157
1,0	< 0,01		< 0,01		< 0,01		23	5	423	74	1,6E+05	1,4E+04
2,0	< 0,01		< 0,01		< 0,01		< 1		61	8	3,1E+04	2,3E+03
3,0	< 0,01		< 0,01		< 0,01		< 1		18	6	1,3E+04	1,2E+03
4,0	< 0,01		< 0,01		< 0,01		< 1		35	6	1,3E+04	1,2E+03
5,0	< 0,01		< 0,01		< 0,01		< 1		73	9	1,7E+04	1,4E+03
6,0	< 0,01		< 0,01		< 0,01		< 1		227	16	1,5E+04	1,3E+03
7,0	0,15	0,04	< 0,01		< 0,01		< 1		84	10	1,7E+04	1,4E+03
8,0	1,97	0,15	0,03	0,03	1,78	1,78	3	2	66	9	1,4E+04	1,3E+03
9,0	0,02	0,02	< 0,01		< 0,01		< 1		4	2	1,3E+04	1,2E+03
11,0	0,02	0,02	< 0,01		< 0,01		< 1		21	5	1,2E+04	1,2E+03
12,0	< 0,01		< 0,01		< 0,01		6	3	3	2	1,4E+04	1,5E+03
13,0	< 0,01		< 0,01		0,01	0,01	< 1		< 1		1,2E+04	1,2E+03
14,0	0,05	0,03	< 0,01		< 0,01		< 1		9	3	1,1E+04	1,1E+03
15,0	< 0,01		< 0,01		0,16	0,16	< 1		< 1		1,2E+04	1,2E+03
16,0	0,03	0,02	< 0,01		0,02	0,02	< 1		< 1		1,1E+04	1,1E+03
16,8	< 0,01		< 0,01		0,01	0,01	2	2	< 1		1,3E+04	1,2E+03
18,0	< 0,01		< 0,01		0,01	0,01	< 1		< 1		1,5E+04	1,3E+03
19,0	< 0,01		< 0,01		0,08	0,08	< 1		< 1		2,6E+04	5,5E+03
20,0	< 0,01		< 0,01		0,01	0,01	< 1		< 1		2,0E+04	1,5E+03
21,0	< 0,01		< 0,01		< 0,01		< 1		2	2	1,3E+04	1,2E+03
22,0	< 0,01		< 0,01		0,05	0,05	2	2	3	2	1,6E+04	1,4E+03
22,8	< 0,01		< 0,01		< 0,01		< 1		1	2	< 10	

Table A 80: Raw data of taken from the spigot of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,0	< 0,01		< 0,01		< 0,01		4	2	10	4	9,6E+03	1,1E+03
2,0	< 0,01		< 0,01		< 0,01		< 1		< 1		2,5E+03	541
3,0	< 0,01		< 0,01		< 0,01		1	2	1	1	2,4E+03	525
4,0	< 0,01		< 0,01		< 0,01		< 1		4	2	2,7E+03	175
5,0	< 0,01		< 0,01		< 0,01		< 1		2	3	1,7E+03	448
6,0	< 0,01		< 0,01		< 0,01		1	2	< 1		5,5E+03	799
7,0	< 0,01		< 0,01		< 0,01		< 1		11	4	548	261
8,0	< 0,01		< 0,01		< 0,01		2	3	22	5	1,9E+03	482
9,0	< 0,01		< 0,01		< 0,01		< 1		< 1		2,4E+03	531
11,0	< 0,01		< 0,01		< 0,01		< 1		122	12	5,3E+03	794
12,0	< 0,01		< 0,01		< 0,01		4	2	23	5	1,3E+03	397
13,0	< 0,01		< 0,01		< 0,01		< 1		4	2	625	274
14,0	< 0,01		< 0,01		< 0,01		< 1		3	2	4,6E+03	743
15,0	< 0,01		< 0,01		< 0,01		< 1		2	1	1,9E+03	482
16,0	< 0,01		< 0,01		< 0,01		< 1		3	2	4,1E+03	690
16,8	< 0,01		< 0,01		< 0,01		< 1		< 1		3,7E+03	653
18,0	< 0,01		< 0,01		< 0,01		< 1		< 1		5,2E+03	779
19,0	< 0,01		< 0,01		< 0,01		< 1		9	3	1,8E+03	142
20,0	< 0,01		< 0,01		< 0,01		< 1		3	3	939	331
21,0	< 0,01		< 0,01		< 0,01		< 1		3	2	4,6E+03	735
22,0	< 0,01		< 0,01		< 0,01		< 1		27	6	1,6E+03	448
22,8	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	

Biofilm samples – Rig Ref

Table A 81: Raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		20	15	790	854
2,0	< 0,06	< 0,06	< 0,06	< 0,6		25	5	3,3E+04	4,9E+03	2,0E+04	1,1E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		26	4	1,5E+04	970	5,2E+05	1,4E+05
10,0	< 0,06	< 0,06	< 0,06	< 0,6		66	7	6,1E+04	6,5E+03	2,5E+05	9,6E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		9	2	1,1E+05	8,6E+03	1,5E+06	2,3E+05
16,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	1,5E+05	1,2E+04	3,1E+05	8,7E+04
22,0	< 0,06	< 0,06	< 0,06	3	3	8	2	4,7E+05	6,0E+04	1,8E+06	5,5E+05
22,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	22	22	< 60	

Table A 82: FISH raw data of biofilm section Bs 1 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 83: Raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		195	435
2,0	< 0,06	< 0,06	< 0,06	1	1	10	3	9,3E+03	779	2,0E+05	7,9E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		12	3	4,7E+03	549	7,8E+05	3,0E+05
10,0	< 0,06	< 0,06	< 0,06	1	2	3	1	4,1E+04	5,1E+03	2,7E+05	4,4E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	1,8E+04	1,3E+03	1,7E+06	3,1E+05
16,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		1,7E+04	3,3E+03	7,6E+05	2,6E+05
22,0	< 0,06	< 0,06	< 0,06	2	3	14	9	9,3E+04	1,4E+04	4,6E+06	1,2E+06
22,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	

Table A 84: FISH raw data of biofilm section Bs 2 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 85: Raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		4	7	244	339
2,0	< 0,06	< 0,06	< 0,06	< 0,6		140	24	1,7E+04	2,7E+03	1,2E+05	3,5E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		7	2	3,2E+03	357	4,3E+05	6,0E+04
10,0	< 0,06	< 0,06	< 0,06	1	1	1	1	8,1E+03	573	1,5E+05	2,7E+04
14,0	< 0,06	< 0,06	< 0,06	1	1	< 0,6		6,9E+03	630	3,1E+05	9,7E+04
16,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		5,7E+03	481	5,0E+05	1,0E+05
22,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	6,4E+03	509	9,5E+05	2,3E+05
22,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	< 6		< 60	

Table A 86: FISH raw data of biofilm section Bs 3 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 87: Raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		61	201
2,0	< 0,06	< 0,06	< 0,06	< 0,6		14	10	1,2E+03	55	1,1E+04	4,9E+03
5,0	< 0,06	< 0,06	< 0,06	< 0,6		17	3	2,3E+03	36	5,0E+04	1,3E+04
10,0	< 0,06	< 0,06	< 0,06	1	1	35	3	2,5E+04	26	1,1E+05	1,4E+04
14,0	< 0,06	< 0,06	< 0,06	1	1	241	32	3,8E+04	35	3,9E+05	9,5E+04
16,9	< 0,06	< 0,06	< 0,06	< 0,6		24	10	8,5E+04	69	3,2E+05	5,5E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	840	106	2,6E+04	156	6,9E+05	1,2E+05
22,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	

Table A 88: FISH raw data of biofilm section Bs 4 taken from Rig Ref at 20°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		4,9	10,0	21,9
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Biofilm samples – Rig Con

Table A 89: Raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		6	11	582	996
2,0	< 0,06	< 0,06	< 0,06	< 0,6		49	6	1,3E+05	9,4E+03	4,3E+05	8,5E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		509	58	4,3E+04	5,3E+03	7,4E+05	1,3E+05
10,0	23,25	< 0,06	< 0,06	< 0,6		7,0E+03	703	6,7E+05	1,0E+05	6,6E+06	1,9E+06
14,0	< 0,06	< 0,06	< 0,06	< 0,6		9,3E+03	801	8,8E+05	7,8E+04	5,2E+06	1,8E+06
16,9	< 0,06	< 0,06	< 0,06	1	1	452	55	1,0E+06	8,1E+04	5,4E+06	4,7E+06
22,0	< 0,06	< 0,06	< 0,06	< 0,6		259	42	1,2E+06	1,1E+05	5,7E+07	1,7E+07
22,9	< 0,06	< 0,06	< 0,06	< 0,6		6	11	11	16	< 60	

Table A 90: FISH raw data of biofilm section Bs 1 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	+	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 91: Raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		1,3E+03	2,4E+03
2,0	< 0,06	< 0,06	< 0,06	< 0,6		85	7	3,7E+04	4,9E+03	2,0E+05	7,2E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		461	56	1,6E+05	1,2E+04	1,2E+06	3,9E+05
10,0	0,86	< 0,06	< 0,06	< 0,6		741	69	4,2E+05	5,2E+04	8,9E+05	3,0E+05
14,0	< 0,06	< 0,06	< 0,06	< 0,6		2,2E+03	205	9,7E+04	1,3E+04	1,8E+06	7,0E+05
16,9	< 0,06	< 0,06	0,06	1	1	979	79	5,1E+05	5,7E+04	2,5E+06	9,3E+05
22,0	< 0,06	< 0,06	0,06	1	2	135	30	5,8E+05	6,3E+04	4,7E+07	4,0E+07
22,9	< 0,06	< 0,06	< 0,06	1	1	< 0,6		< 0,6		< 60	

Table A 92: FISH raw data of biofilm section Bs 2 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 93: Raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		202	325
2,0	< 0,06	< 0,06	< 0,06	< 0,6		199	29	2,9E+04	3,5E+03	1,4E+05	3,4E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		479	45	1,3E+04	2,3E+03	3,7E+05	9,4E+04
10,0	0,08	< 0,06	< 0,06	< 0,6		73	15	3,7E+04	3,3E+03	1,6E+05	5,7E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		41	4	5,8E+04	4,9E+03	2,7E+06	1,3E+06
16,9	< 0,06	< 0,06	< 0,06	1	1	89	7	1,5E+05	2,5E+04	8,0E+05	4,7E+05
22,0	< 0,06	< 0,06	< 0,06	1	1	73	6	7,6E+04	6,8E+03	2,8E+06	1,5E+06
22,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	< 6		< 60	

Table A 94: FISH raw data of biofilm section Bs 3 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 95: Raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,3	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		66	209
2,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	710	54	4,0E+04	1,3E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		27	3	335	37	8,5E+04	1,2E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		2	1	370	38	5,7E+04	1,3E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		5	1	283	35	4,3E+04	7,8E+03
16,9	< 0,06	< 0,06	< 0,06	< 0,6		7	2	893	72	2,6E+05	7,4E+04
22,0	< 0,06	< 0,06	< 0,06	1	1	1	1	600	157	2,7E+05	1,1E+05
22,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 0,6		< 0,6	

Table A 96: FISH raw data of biofilm section Bs 4 of Rig Con at 20°C operating temperature and dosage of 300 µg/L Na-acetate (TS 6)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

10.7 Raw data of TS 7

Water samples – Rig Ref

Table A 97: Raw data of tank water taken from Rig Ref at 36°C operating temperature and dosage of 100 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa* WAM	± 95 % CI	*E. coli* WAM	± 95 % CI	*E. faecalis* WAM	± 95 % CI	CC 20°C WAM	± 95 % CI	CC 36°C WAM	± 95 % CI	HPC WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
0,8	< 0,01		< 0,01		< 0,01		< 1		125	39	2,7E+04	2,1E+03
1,8	< 0,01		< 0,01		< 0,01		< 1		424	70	3,1E+04	2,2E+03
2,8	< 0,01		< 0,01		< 0,01		< 1		2,0E+03	152	2,0E+04	1,5E+03
3,8	< 0,01		< 0,01		< 0,01		< 1		196	18	2,0E+04	1,6E+03
4,8	< 0,01		< 0,01		< 0,01		1	1	176	17	1,9E+04	1,5E+03
5,8	< 0,01		< 0,01		< 0,01		1	2	49	8	1,1E+04	1,1E+03
6,8	< 0,01		< 0,01		< 0,01		< 1		128	12	8,5E+03	994
7,8	< 0,01		< 0,01		< 0,01		< 1		223	16	1,0E+04	1,1E+03
8,8	< 0,01		< 0,01		< 0,01		< 1		40	23	1,6E+04	1,4E+03
9,8	< 0,01		< 0,01		< 0,01		< 1		35	26	1,4E+04	1,3E+03
10,8	< 0,01		< 0,01		< 0,01		1	2	23	5	6,6E+03	876
11,8	< 0,01		< 0,01		< 0,01		< 1		93	11	5,8E+03	816
12,8	< 0,01		< 0,01		< 0,01		< 1		81	10	8,4E+03	989
13,8	< 0,01		< 0,01		< 0,01		< 1		94	11	1,3E+04	1,2E+03
14,8	< 0,01		< 0,01		< 0,01		< 1		105	11	1,7E+04	1,4E+03
15,8	< 0,01		< 0,01		< 0,01		< 1		85	10	9,2E+03	1,0E+03
16,8	< 0,01		< 0,01		< 0,01		< 1		124	12	9,6E+03	1,3E+03
17,8	< 0,01		< 0,01		< 0,01		< 1		42	23	1,0E+04	1,1E+03
18,8	< 0,01		< 0,01		< 0,01		1	2	176	15	6,7E+03	878
19,8	< 0,01		< 0,01		< 0,01		< 1		198	24	6,6E+03	873
20,8	< 0,01		< 0,01		< 0,01		1	2	288	58	4,5E+03	723
21,8	< 0,01		< 0,01		< 0,01		2	1	220	16	7,3E+03	919
22,7	< 0,01		< 0,01		< 0,01		4	3	2	2	< 10	

Water samples – Rig Con

Table A 98: Raw data of tank water taken from Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa* WAM	± 95 % CI	*E. coli* WAM	± 95 % CI	*E. faecalis* WAM	± 95 % CI	CC 20°C WAM	± 95 % CI	CC 36°C WAM	± 95 % CI	HPC WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		1	2	< 10	
0,8	< 0,01		< 0,01		< 0,01		< 1		112	12	1,2E+05	1,2E+04
1,8	< 0,01		< 0,01		< 0,01		< 1		1,1E+03	111	1,7E+05	1,7E+04
2,8	< 0,01		< 0,01		< 0,01		< 1		755	94	1,1E+05	1,1E+04
3,8	< 0,01		< 0,01		< 0,01		< 1		184	47	1,0E+05	1,1E+04
4,8	< 0,01		< 0,01		< 0,01		< 1		622	86	8,9E+04	1,0E+04
5,8	< 0,01		< 0,01		< 0,01		< 1		109	11	8,6E+04	1,0E+04
6,8	< 0,01		< 0,01		< 0,01		1	2	364	65	5,5E+04	8,0E+03
7,8	< 0,01		< 0,01		< 0,01		1	2	136	40	8,9E+04	1,0E+04
8,8	< 0,01		< 0,01		< 0,01		< 1		150	21	1,3E+05	1,2E+04
9,8	< 0,01		< 0,01		< 0,01		< 1		341	64	1,4E+05	1,2E+04
10,8	< 0,01		< 0,01		< 0,01		1	2	78	31	7,6E+04	9,4E+03
11,8	< 0,01		< 0,01		< 0,01		< 1		32	20	5,6E+04	8,1E+03
12,8	< 0,01		< 0,01		< 0,01		< 1		94	33	8,2E+04	9,8E+03
13,8	< 0,01		< 0,01		< 0,01		< 1		218	29	1,3E+05	1,2E+04
14,8	< 0,01		< 0,01		< 0,01		< 1		223	53	1,9E+05	1,5E+04
15,8	< 0,01		< 0,01		< 0,01		1	2	186	18	8,0E+04	9,6E+03
16,8	< 0,01		< 0,01		< 0,01		< 1		382	67	1,8E+05	1,5E+04
17,8	< 0,01		< 0,01		< 0,01		< 1		448	75	1,4E+05	1,6E+04
18,8	< 0,01		< 0,01		< 0,01		< 1		253	21	1,0E+05	1,1E+04
19,8	< 0,01		< 0,01		< 0,01		< 1		348	66	7,5E+04	9,3E+03
20,8	< 0,01		< 0,01		< 0,01		1	2	150	21	1,0E+05	1,3E+04
21,8	< 0,01		< 0,01		< 0,01		4	2	1,4E+03	126	1,5E+05	1,3E+04
22,7	< 0,01		< 0,01		< 0,01		3	2	2	2	< 10	

Table A 99: Raw data of taken from the faucet of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		1	2	< 10	
0,8	< 0,01		< 0,01		< 0,01		< 1		68	9	3,6E+04	6,5E+03
1,8	< 0,01		< 0,01		< 0,01		< 1		712	91	6,2E+04	8,5E+03
2,8	< 0,01		< 0,01		< 0,01		< 1		476	74	7,1E+04	9,1E+03
3,8	< 0,01		< 0,01		< 0,01		< 1		239	20	7,3E+04	9,2E+03
4,8	< 0,01		< 0,01		< 0,01		< 1		458	73	5,6E+04	8,1E+03
5,8	< 0,01		< 0,01		< 0,01		< 1		107	11	7,3E+04	9,2E+03
6,8	0,06	0,03	< 0,01		0,01	0,01	< 1		14	4	4,6E+04	7,3E+03
7,8	0,29	0,06	< 0,01		0,08	0,03	4	4	448	72	5,2E+04	7,7E+03
8,8	0,10	0,04	< 0,01		0,03	0,02	< 1		240	26	6,2E+04	8,6E+03
9,8	0,05	0,03	< 0,01		0,11	0,04	< 1		891	102	8,3E+04	9,8E+03
10,8	0,04	0,02	< 0,01		0,93	0,10	1	2	70	28	5,2E+04	7,9E+03
11,8	< 0,01		< 0,01		< 0,01		1	2	1,3E+03	123	2,2E+04	5,2E+03
12,8	0,10	0,03	< 0,01		0,18	0,05	< 1		22	16	8,7E+04	3,8E+04
13,8	0,05	0,02	< 0,01		< 0,01		< 1		172	17	8,9E+04	1,0E+04
14,8	0,24	0,05	< 0,01		0,27	0,06	< 1		35	21	1,4E+05	1,3E+04
15,8	0,16	0,04	< 0,01		0,11	0,04	< 1		201	18	8,1E+04	9,7E+03
16,8	0,02	0,02	< 0,01		1,17	0,12	< 1		288	58	6,8E+04	8,8E+03
17,8	0,76	0,09	< 0,01		1,99	0,15	2	2	216	52	1,1E+05	1,4E+04
18,8	0,02	0,02	< 0,01		0,01	0,01	< 1		199	15	5,3E+04	7,8E+03
19,8	18,39	1,46	< 0,01		0,73	0,09	1	2	252	54	6,4E+04	8,6E+03
20,8	2,52	0,54	< 0,01		0,04	0,02	1	1	185	18	7,2E+04	9,1E+03
21,8	17,48	1,43	< 0,01		5,85	0,83	33	7	280	28	1,2E+05	1,2E+04
22,7	< 0,01		< 0,01		< 0,01		3	2	3	2	< 10	

Table A 100: Raw data of taken from the spigot of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*		*E. coli*		*E. faecalis*		CC 20°C		CC 36°C		HPC	
	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL		cfu/mL	
-0,3	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
0,8	< 0,01		< 0,01		< 0,01		< 1		< 1		< 10	
1,8	< 0,01		< 0,01		< 0,01		< 1		4	2	313	60
2,8	< 0,01		< 0,01		< 0,01		< 1		259	17	4,4E+03	713
3,8	< 0,01		< 0,01		< 0,01		< 1		244	17	6,2E+03	846
4,8	< 0,01		< 0,01		< 0,01		< 1		189	15	2,3E+03	527
5,8	< 0,01		< 0,01		< 0,01		< 1		22	5	1,6E+04	1,3E+03
6,8	< 0,01		< 0,01		< 0,01		< 1		463	77	2,3E+03	521
7,8	< 0,01		< 0,01		< 0,01		< 1		19	5	9,7E+03	1,4E+03
8,8	< 0,01		< 0,01		< 0,01		< 1		251	17	1,8E+04	1,4E+03
9,8	< 0,01		< 0,01		< 0,01		< 1		75	9	2,5E+03	541
10,8	< 0,01		< 0,01		< 0,01		< 1		75	9	2,6E+03	555
11,8	< 0,01		< 0,01		< 0,01		< 1		2	2	1,9E+03	471
12,8	< 0,01		< 0,01		< 0,01		< 1		17	4	2,0E+03	483
13,8	< 0,01		< 0,01		< 0,01		< 1		1	1	1,7E+04	1,4E+03
14,8	< 0,01		< 0,01		< 0,01		< 1		10	4	2,4E+04	1,7E+03
15,8	< 0,01		< 0,01		< 0,01		< 1		14	4	1,4E+03	415
16,8	< 0,01		< 0,01		< 0,01		< 1		71	9	2,4E+03	667
17,8	< 0,01		< 0,01		< 0,01		< 1		18	5	1,9E+04	1,5E+03
18,8	< 0,01		< 0,01		< 0,01		< 1		23	5	1,5E+04	1,3E+03
19,8	< 0,01		< 0,01		< 0,01		< 1		10	4	2,5E+03	544
20,8	< 0,01		< 0,01		< 0,01		1	2	3	2	1,1E+03	369
21,8	< 0,01		< 0,01		< 0,01		< 1		8	3	1,4E+03	410
22,7	< 0,01		< 0,01		< 0,01		3	2	2	2	< 10	

Biofilm samples – Rig Ref

Table A 101: Raw data of biofilm section Bs 1 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		1	1	22	22	< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		134	30	6,7E+04	6,6E+03	3,9E+05	9,3E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		329	48	9,4E+04	8,0E+03	4,8E+05	9,3E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		391	52	9,4E+04	8,0E+03	1,9E+05	4,9E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		7	3	2,0E+04	3,7E+03	4,5E+05	6,3E+04
18,0	< 0,06	< 0,06	< 0,06	< 0,6		12	3	8,3E+04	7,6E+03	4,3E+06	2,6E+06
22,0	< 0,06	< 0,06	< 0,06	1	1	33	4	7,3E+04	6,4E+03	1,2E+06	3,4E+05
22,9	< 0,06	< 0,06	< 0,06	1	1	1	1	< 6		1,1E+04	2,8E+03

Table A 102: FISH raw data of biofilm section Bs 1 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		4,9	10,0	21,9
Non EUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 103: Raw data of biofilm section Bs 2 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		79	7	6,6E+03	2,0E+03	6,1E+05	1,6E+05
5,0	< 0,06	< 0,06	< 0,06	< 0,6		1,1E+03	86	6,1E+04	6,5E+03	3,8E+05	5,1E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		239	40	1,4E+04	973	3,6E+05	1,2E+05
14,0	< 0,06	< 0,06	< 0,06	< 0,6		4	2	5,2E+03	585	3,3E+05	8,5E+04
18,0	< 0,06	< 0,06	< 0,06	1	1	5	2	5,0E+03	697	7,8E+05	2,3E+05
22,0	< 0,06	< 0,06	< 0,06	1	1	10	2	8,3E+03	684	1,5E+05	4,5E+04
22,9	< 0,06	< 0,06	< 0,06	1	1	< 0,6		< 6		7,6E+03	3,5E+03

Table A 104: FISH raw data of biofilm section Bs 2 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		4,9	10,0	21,9
Non EUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 105: Raw data of biofilm section Bs 3 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		1	1	< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		38	4	1,4E+04	2,4E+03	1,3E+05	4,0E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		94	6	3,9E+04	4,0E+03	2,6E+05	5,3E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		59	5	1,1E+04	2,1E+03	1,9E+05	1,0E+05
14,0	< 0,06	< 0,06	< 0,06	< 0,6		3	1	6,0E+03	498	3,5E+05	1,1E+05
18,0	< 0,06	< 0,06	< 0,06	< 0,6		20	3	1,5E+04	2,5E+03	5,7E+05	2,6E+05
22,0	< 0,06	< 0,06	< 0,06	1	1	9	2	2,2E+04	3,1E+03	1,4E+05	2,7E+04
22,9	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		6,4E+03	2,2E+03

Table A 106: FISH raw data of biofilm section Bs 3 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		4,9	10,0	21,9
Non EUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 107: Raw data of biofilm section Bs 4 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

week of	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
operation	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		9	2	829	25	1,8E+04	8,4E+03
5,0	< 0,06	< 0,06	< 0,06	1	1	13	2	162	346	6,0E+04	1,1E+04
10,0	< 0,06	< 0,06	< 0,06	< 0,6		4	1	698	156	4,0E+04	1,1E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		8	6	5,7E+03	312	7,2E+04	2,6E+04
18,0	< 0,06	< 0,06	< 0,06	< 0,6		21	9	3,5E+03	233	3,8E+05	1,0E+05
22,0	< 0,06	< 0,06	< 0,06	< 0,6		253	32	5,2E+03	38	3,5E+04	1,4E+04
22,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	< 6		2,2E+03	1,7E+03

Table A 108: FISH raw data of biofilm section Bs 4 taken from Rig Ref at 36°C operating temperature and a dosage of 100 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		4,9	10,0	21,9
Non EUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Biofilm samples – Rig Con

Table A 109: Raw data of biofilm section Bs 1 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		6	11	11	16	< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		3,0E+03	423	5,6E+04	5,5E+03	5,5E+05	1,8E+05
5,0	< 0,06	< 0,06	< 0,06	1	1	6,0E+04	6,6E+03	8,3E+05	7,4E+04	2,3E+06	5,8E+05
10,0	< 0,06	< 0,06	< 0,06	< 0,6		1,0E+05	8,2E+03	3,2E+05	4,7E+04	1,1E+06	3,3E+05
14,0	< 0,06	0,06	0,17	< 0,6		104	145	6,4E+05	6,5E+04	7,1E+07	3,6E+07
18,0	< 0,06	< 0,06	0,06	1	1	72	8	3,0E+05	4,6E+04	4,1E+07	1,7E+07
22,0	< 0,06	< 0,06	< 0,06	2	1	141	34	9,0E+04	7,1E+03	5,2E+07	2,0E+07
22,9	< 0,06	< 0,06	< 0,06	1	1	< 0,6		< 6		1,6E+04	5,3E+03

Table A 110: FISH raw data of biofilm section Bs 1 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 111: Raw data of biofilm section Bs 2 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	1	1	< 0,6		< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	1	1	3,0E+03	468	1,8E+05	1,7E+04	6,6E+05	1,6E+05
5,0	< 0,06	< 0,06	< 0,06	1	1	4,8E+04	5,9E+03	2,9E+05	4,5E+04	3,5E+06	1,7E+06
10,0	< 0,06	< 0,06	< 0,06	< 0,6		3,0E+04	4,5E+03	1,2E+06	9,2E+04	4,3E+07	1,2E+07
14,0	< 0,06	< 0,06	0,24	< 0,6		2,3E+03	478	5,9E+04	7,5E+03	2,2E+06	5,1E+05
18,0	< 0,06	< 0,06	0,06	1	1	1,8E+03	368	6,8E+04	6,8E+03	5,3E+05	2,7E+05
22,0	< 0,06	0,05	< 0,06	4	2	123	27	7,6E+04	6,5E+03	9,4E+06	4,3E+06
22,9	< 0,06	< 0,06	< 0,06	1	1	1	1	< 6		1,2E+04	5,0E+03

Table A 112: FISH raw data of biofilm section Bs 2 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 113: Raw data of biofilm section Bs 3 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		394	40	3,1E+04	3,5E+03	2,8E+05	5,3E+04
5,0	< 0,06	< 0,06	< 0,06	< 0,6		4,1E+03	403	4,0E+04	3,9E+03	4,3E+05	1,4E+05
10,0	< 0,06	< 0,06	< 0,06	< 0,6		1,9E+03	281	3,8E+04	3,9E+03	3,5E+05	5,9E+04
14,0	< 0,06	< 0,06	< 0,06	< 0,6		46	14	2,0E+04	2,7E+03	5,7E+05	1,7E+05
18,0	< 0,06	< 0,06	< 0,06	2	1	14	2	2,9E+04	3,3E+03	3,1E+05	2,5E+05
22,0	< 0,06	< 0,06	< 0,06	2	1	46	4	3,0E+04	3,5E+03	3,1E+06	1,9E+06
22,9	< 0,06	< 0,06	< 0,06	1	1	< 0,6		< 6		5,7E+03	3,0E+03

Table A 114: FISH raw data of biofilm section Bs 3 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Table A 115: Raw data of biofilm section Bs 4 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

week of operation	*P. aeruginosa*	*E. coli*	*E. faecalis*	CC 20°C		CC 36°C		HPC		TCC	
				WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI	WAM	± 95 % CI
	cfu/cm²	cfu/cm²	cfu/cm²	cfu/cm²		cfu/cm²		cfu/cm²		cells/cm²	
-0,1	< 0,06	< 0,06	< 0,06	< 0,6		< 0,6		< 6		< 60	
2,0	< 0,06	< 0,06	< 0,06	< 0,6		18	3	124	27	2,8E+04	7,0E+03
5,0	< 0,06	< 0,06	< 0,06	< 0,6		146	24	3,0E+03	346	1,4E+04	9,0E+03
10,0	< 0,06	< 0,06	< 0,06	< 0,6		1	1	576	163	4,6E+04	5,6E+03
14,0	< 0,06	< 0,06	< 0,06	< 0,6		9	2	2,5E+03	326	2,0E+04	6,8E+03
18,0	< 0,06	< 0,06	< 0,06	< 0,6		4	2	1,3E+03	233	866	945
22,0	< 0,06	< 0,06	< 0,06	2	1	2	1	374	39	1,3E+03	1,2E+03
22,9	< 0,06	< 0,06	< 0,06	< 0,6		1	1	< 6		583	644

Table A 116: FISH raw data of biofilm section Bs 4 of Rig Con at 36°C operating temperature and dosage of 300 µg/L Na-acetate (TS 7)

Probe	Target	week of operation		
		5,0	10,0	22,0
NONEUB	unspecific binding	-	-	-
Psae16S-182	*P. aeruginosa*	-	-	-

Percent of TCC: - not detected; + n < 1%; ++ 1 % < n < 10 %; +++ n > 10 %

Curriculum Vitae

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