METHODOLOGY OF MODELING BACTERIAL GROWTH IN DRINKING WATER SYSTEMS

TECHNEAU

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Summary

The report is aiming to critically review available bacterial regrowth models in water distribution and to propose the conceptual model for further use in TECHNEAU project.

The first section presents the literature review about links of water quality problems (coliform, chorine residual loss) to bacterial regrowth. The major models available are presented in the second section. The trade-offs between simple statistical models and more complex mathematical models are discussed. In the third section the mathematic model developed in TECHNEAU project are available.

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Abbreviations

AOC	assimilable organic carbon without addition of inorganic nutrients
BDOC	biologically degradable organic carbon
BDOCsusp	BDOC measured with technique with suspended biomass
BDOCsand	BDOC measured with technique with attached biomass
BOM	biodegrable organic matter
C	carbon
CFU	bacterial colony forming unit
DAPI	4',6-Diamidino-2-Phenylindole
DBPs	disinfection by-products
DOC	dissolved organic carbon (< 0.45 µm filtrate)
DNA	deoxyribonucleic acid
DS	distribution system
DWD	Drinking Water Directive 98/83/EC
EFTA	European Free Trade Association
EC	European Commission
EU	European Union
GAC	granular activated carbon
GDWR	German drinking water regulations methods
HPC	heterotrophic plate count
HS	humic substances
IMSL	software library used in computer programming language FORTRAN
NF	nanofiltration
NOM	natural organic matter
PE	polyethylene
PF	plug flow
PMWR	perfectly mixed with water recycling
PVC	polyvinylchloride
RNA	ribonucleic acid
TOC	total organic carbon
TBN	total bacterial number
TM	trade mark
TZW	Technologiezentrum Wasser
USA	United States of America
VNBC	viable but not-cultivable
WEKNOW	EC 5th Framework Programme "Web-based European Knowledge Network on Water", contract no. EVK1-CT-2002-2004

1 MICROBIOLOGICAL PARAMETERS FOR DRINKING WATER QUALITY ASSESMENT

1.1 Microbiological parameters in European Union Drinking Water Directive 98/83/EC

Within European Union (EU) Member States, candidate countries and European Free Trade Association (EFTA) countries drinking water should meet for the Drinking Water Directive 98/83/EC which intends that water should be wholesome and clean; it should be free from any microorganisms and parasites and from any substances which, in number or concentration, constitute a potential danger to human health. The microbial quality in the DWD is addressed by setting maximal allowed limits of human gastroenteric bacteria *Eschericia coli* (*E.coli*) and Enterococci. Concentration of these microorganisms should not exceed 0 cells in 100 mL of water sample. Spore forming bacteria *Clostridium perfringens* is a parameter which should be analysed in some specific conditions, namely, after distribution main disinfection and in distribution systems (DS), which are supplied by surface waters.
In the DWD heterotrophic plate count (HPC) is used as a parameter to control bacteria number in bottled water. However, many EU countries have adapted HPC also for drinking water control. The maximal allowed value is 100 colony forming units (CFU)/mL after water cultivation on media for 3 days at 22^{o}C.

1.2 Additional microbiological parameters within countries of European Union and wide-world

Some EU countries have introduced more strengthened requirements for microbiological quality of drinking water. For example HPC is routine parameter in Cyprus, Czech Republic, Estonia, Germany, Hungary, Latvia, Malta, the Netherlands, Poland, Romania, Slovak Republic, Slovenia, and Switzerland. The maximal concentration is 100 CFU/mL. In Northern America and Canada the guideline for HPC are 500 CFU/mL (Letterman, 1999).

In Slovak Republic, iron and manganese bacteria, and *Flagellata apochromata* are controlled, in Czech Republic - *Legionella*; in Italy algae, specific bacteriophage, helminths, pathogenic enterobacteria, enterovirus, fungi, protozoa, *Pseudomonas aeruginosa,* and pathogenic Staphylococci; in England and Wales - cryptosporidium oocysts (EC, WEKNOW, project newsletters, 2003-2005), in the Netherlands *Aeromonas*.

Furthermore, this report will be focused of two parameters: coliforms (as well *E.coli*) and HPC, because they are related to bacteria regrowth.

1.3 Shortages of HPC and coliform methods

The conventional methods used for enumerations of HPC (Reasoner and Geldreich, 1985) have many disadvantages. In plate count procedure, the organisms are enumerated on a relatively restricted range of culture media and incubation conditions are not appropriate for many of the organisms present in drinking water. In addition, it has been shown that the recovery of heterotrophic bacteria by these methods varies greatly with the medium and with the plating technique used. Partially, the lack of cultivability may be due to the presence of starved bacteria or those injured by disinfectants. To optimize conditions (reducing nutrient shock) for recovering of starved, slowly growing (Reasoner and Geldreich, 1985) or injured bacteria (McFeeters, 1990) a new, more oligotrophic media has been developed and incubation period was extended to 7 days. This resulted in the enumeration of a larger fraction of the total number of organisms but still only 0.1 to 10% of total bacteria number (TBN) present in the water (Block, 1992). It has also been shown that poor nutrient conditions and the presence of disinfectants in drinking water DSs may cause a large portion of the organisms to enter the viable but non-cultivable state (Byrd *et al*., 1991; Roszak and Colwell, 1987). These bacteria are not detected with HPC methods.
TBN is widely used in research labs for prediction of water microbiological quality. TBN is detected with epifluorescence microscope or cytometer after bacteria staining with a non-specific fluorochrome. This stain typically binds to deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA) and, therefore will allow for detection of all uninjured organisms, regardless of their cultivability. Initially, acridine orange was used (Hobbie *et al*., 1977), but now the most commonly used stain for drinking water applications is 4',6-Diamidino-2-Phenylindole (DAPI) (Brunk *et al.*, 1979, Porter and Feig, 1980) and asymmetrical cyanine dyes SYBR Green (Myers, 1998) and SYBR Gold TM.

The detection of coliforms in tap water is intended to indicate to end-users that an interruption of drinking water treatment process or post contamination during supply has occurred. However, coliforms are sometimes detected when there is no evidence of contamination and they are sometimes not detected when pathogenic organisms are found in the drinking water. It has been reported that during a ten year period in the United States 64% of all waterborne illness outbreaks by bacteria and viruses were associated with coliforms (Craun *et al*., 1997). Much rather (35%) coliforms were detected when outbreaks were caused by protozoa.
Coliforms may also remain undetected as a result of the cultivation techniques used for coliform detection. Some or all of the coliforms may be injured and not killed by disinfection and, thus, unable to grow on traditional agar media. Due to proportionally small amount of water analyzed from all amount of water supplied to costumer routine analyses may not sufficiently safe to indicate to potential health risk.
Survey about analytical methods used for drinking water analyses within WEKNOW project have concluded (2005) that there are several problems with the applying methods for *E.coli*, coliforms and *Clostridium perfringens*

detection of drinking water pollution. Herson *et al*., (1991) have argued that these standard procedures for determination water quality are based on methods that were developed using unattached bacteria thus might not be entirely representative for water distribution networks.

1.4 Factors influencing bacterial regrowth

In general, bacterial growth in drinking water DSs depends on the concentration of organic and inorganic nutrients, water temperature, disinfection residual, sediments and flow velocity. In further subchapters the factors which may influence bacterial growth within DS are discussed; there are showed correlations between these factors and water microbiological parameters (TBN, HPC, *E.coli*, coliforms); thresholds for the significant parameters are given which can ensure microbiologically stable drinking water.

1.4.1 Temperature

Drinking water temperature perhaps is the most important parameter which influences regrowth processes. There are many authors which most often noted that water temperature above 15 ^{o}C significantly increases bacterial regrowth (LeChevallier, 1990). Fransolet *et al*., (1985) found that water temperature influenced not only the growth rate of microorganisms, but the lag phase and cell yield as well. At low temperatures, cells would be washed out of the DS before significant growth could be achieved.
E.coli and other coliform bacteria are known as mesophilic, with growth occurring at 5-45 ^{o}C. Fransolet *et al*., (1985) found that growth of *E.coli* was very slow (growth rates < 0.1 divisions per hour) below 20 ^{o}C. In most of the reported cases, more coliform occurrences have been noted in DSs during summer months when water temperature is at their highest level. According to studies of LeChevallier *et al*., (1991) on DS, the coliform occurrences were most frequent during the summer and fall. They stated that coliform bacteria were not recovered during the winter months and occurred only at low levels during most of the springs, therefore most of these events can be associated with water temperature greater than 15 ^{o}C (Figure 1).

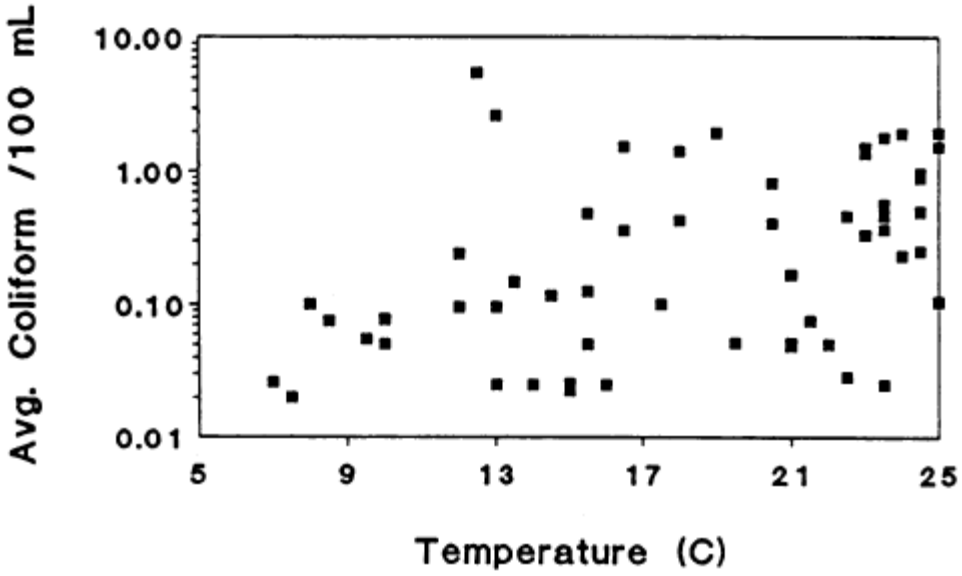

Figure 1. Relationship between temperature and average coliform occurrence (LeChevallier *et al*., 1991)

Another field scale experiment from the city of Montreal in Canada showed that 94% of the positive total (nonfecal) coliform events occurred in DS when the water temperature was above 15 ^{o}C. The lowest water temperature at which total (nonfecal) coliforms were recorded was 12 ^{o}C, and the percentage of positive events increased with temperature (Figure 2) . Each time coliforms were identified, HPC organisms were also detected. In comparison, an analysis by Volk and Joret (1994) of two distribution networks in a suburb of Paris, France, found that 76% of the positive coliform events occurred at water temperatures above 15 ^{o}C (Besner *et al*., 2001). The trends in the occurrence of HPC events showed in Figure 2 as a function of water temperature differed somewhat from the trends observed for total coliforms. In the percentage of samples containing HPC events, two peaks were identified; from 0 to 7.5 ^{o}C and from 12.6 to 22.5 ^{o}C. To explain the higher occurrence of HPC events at lower water temperature, the authors looked at similar events at the treatment plant effluent and stated that applied contact time of disinfectant was too short. The second peak of HPC could be explained by bacterial regrowth processes in network.

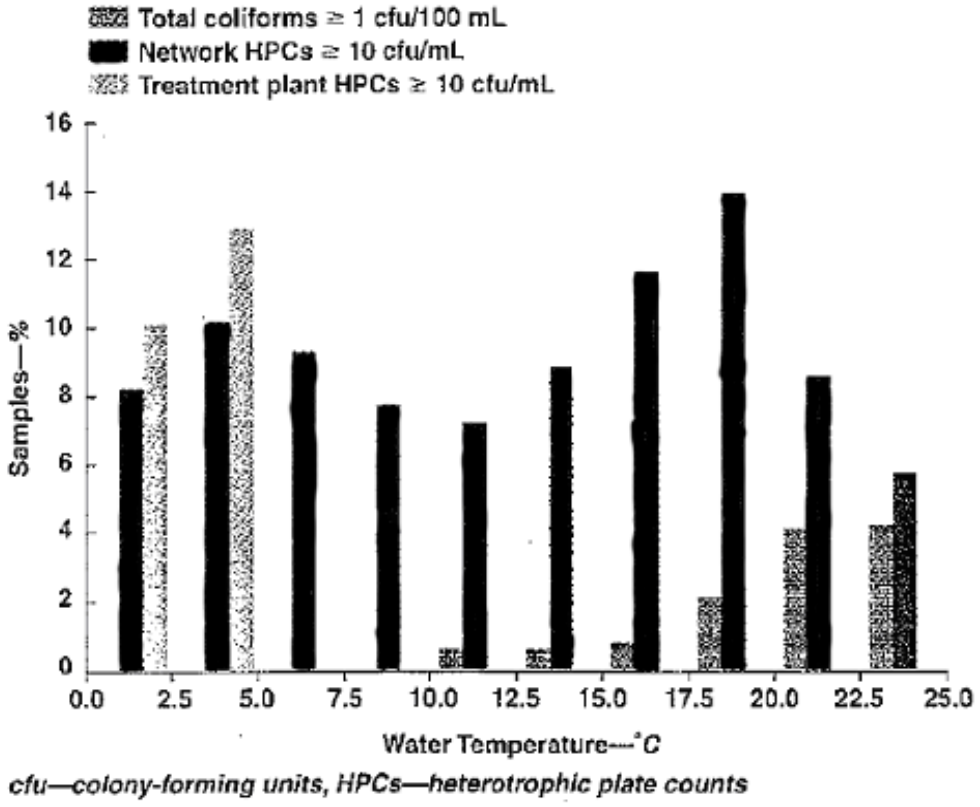

Figure 2. Percentage of samples with positive total coliforms and HPC bacteria respected to water temperature (Besner *et al*., 2001)

1.4.2 Nutrients

In drinking water the limiting nutrient for the bacterial growth is usually the biodegradable fraction of dissolved organic carbon (DOC), even though the limitation of bacterial growth because of the availability of phosphorus has also been shown in Nordic regions, such as Baltic countries, Nordic countries, Japan and North America where drinking water sources are rich with humic substances (HS) (Miettinen, 1997; Sathasivan *et al.,* 1997, Juhna, 2002) and where phosphorus is effectively removed during treatment process by conventional coagulation-sedimentation technology.

The recent studies by Juhna *et al.*, (2007) showed that higher concentrations of phosphorus in drinking water increased the cultivability of *E.coli* in biofilms of water DSs.

The biodegradable fraction of DOC is usually expressed as assimilable organic carbon (AOC) or biodegradable DOC (BDOC) concentration, reflecting two different approaches of analytical procedures. The AOC bioassay is a technique by which the growth of test organism(s) is correlated with the concentration of biodegradable organic matter (BOM). The BDOC assay consists of measuring the consumption of DOC through the ability of a mixed microflora to catabolise organic carbon to carbon dioxide and/or new biomass. This portion of carbon measured as AOC or BDOC is a small pool

from total organic carbon (TOC) concentration varying from 0.1-9% (van der Kooij *et al*., 1990) and 10-30% (Joret *et al*., 1991), respectively for AOC and BDOC. AOC represents the most readily degradable fraction of BDOC/BOM. The drawbacks of AOC measurements assays are time consume and the requirement of a high level of expertise. Also, the use of selected pure cultures supplies limited information for measuring a complex pool of natural bioavailable carbon compounds . A recently proposed method by Hammes and Egli (2005) significantly decreases time necessary for routine AOC measurements. The principal approach proposed by them is the usage of fluorescence staining of total nucleic acids combined with flow cytometry as a rapid and straightforward growth enumeration method compared with plating technique.

The drawback of BDOC method is the low detection limit: 0.1-0.2 mg/L. Conventionally are stated that both, AOC and BDOC concentrations decrease with increases of water residence time in DSs, which hypothetically is assumed by carbon utilization by bacteria and subsequent growth. Thus, bacterial regrowth may be limited by decreasing AOC and BDOC concentration in water leaving the treatment plant.

It has been possible to establish relationship between the concentration of organic matter and the bacterial biomass in the DS. Mathieu *et al*., (1992) have observed, in the absence of disinfection residual, a log-linear relationship between the density of the bacterial cells attached or in suspension and the amount of BDOC in a pipe loop DS (Figure 3.)

To study the effect of BDOC on bacterial dynamic without the interference of chlorine, Servais *et al*., (1992, 1993, 1995) have analyzed data from situations found in several full-scale French DSs, in the absence of disinfectant residual. The results collected covered a wide range of situations in terms of BDOCsusp in water at treatment plants outflows (from 0.1 to 0.7 mg C/L). The mean concentration of fixed bacteria in the DSs studied varied from 3'000'000 to 26'000'000 cells/cm^2. When these values were plotted as a function of the BDOC in the finished water (Figure 4.), a correlation was obtained, that suggests, in the absence of chlorine residual, the amount of BDOC entering the DSs is a major factor controlling the concentration of biofilm bacteria.

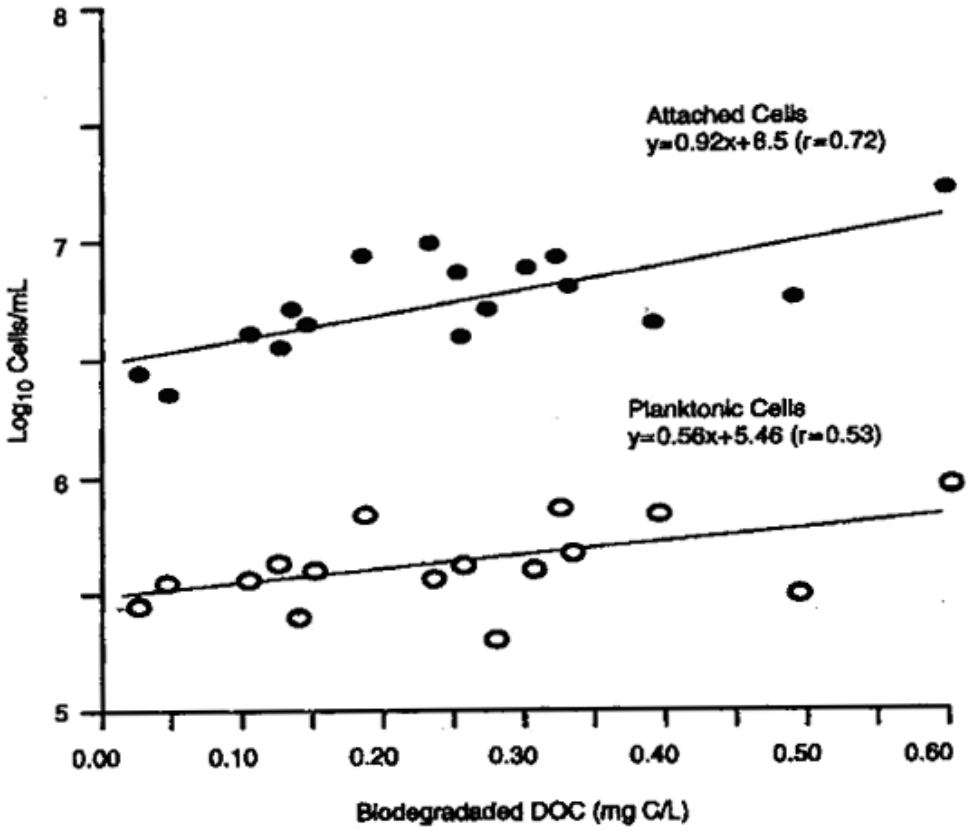

Figure 3. Relationship between the density of attached and planktonic cells (estimated by epifluorescence microscopy) and the concentration of BDOC in the drinking water DS (Mathieu *et al.*, 1992).

Note: 100 mm pipe diameter; temperature 20 °C; absence of disinfectant residual

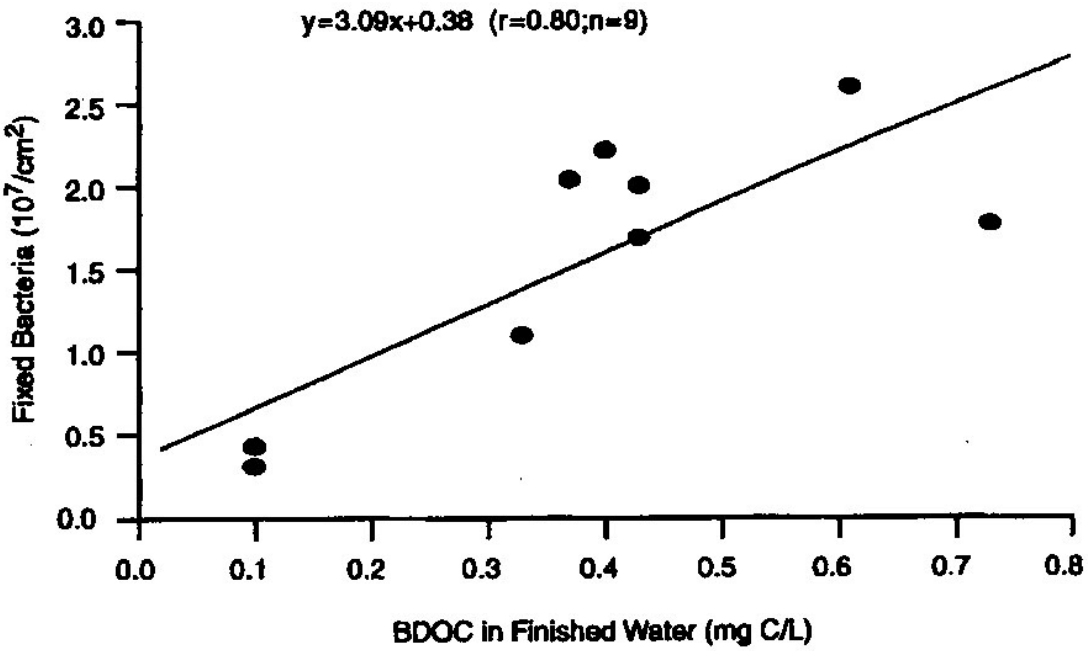

Figure 4. Relationship between average concentration of biofilm bacteria and BDOC in finished water for the various DSs (Servais *et al.*, 1992)

These results are in accordance with those obtained by Volk and LeChevallier (1999) on annular reactors installed in two American drinking water system facilities. The study showed that biofilm densities were related to the amount of biodegradable material entering the system measured as AOC and BDOC. The decrease in nutrient concentrations following implementation of biological filtration resulted in lower biofilm densities over a period of several months (0.5 to 1-log unit reduction).
Moreover, LeChevallier *et al*. (1987) found that growth of *E.coli* isolate was inhibited by AOC levels <54 µg/L and stated on other studies (1991) that the regrowth of coliform bacteria in chlorinated water may be limited by AOC levels of less than 50 to 100 µg/L (Figure 5). For these data, the AOC values were calculated as level which occurred in the treatment plant effluent 7 days earlier. During the wide study in DS he found that average density was 0.44 coliforms/100 mL and daily treatment plant effluent analyses for coliforms on both m-Endo and m-T7 media were negative.

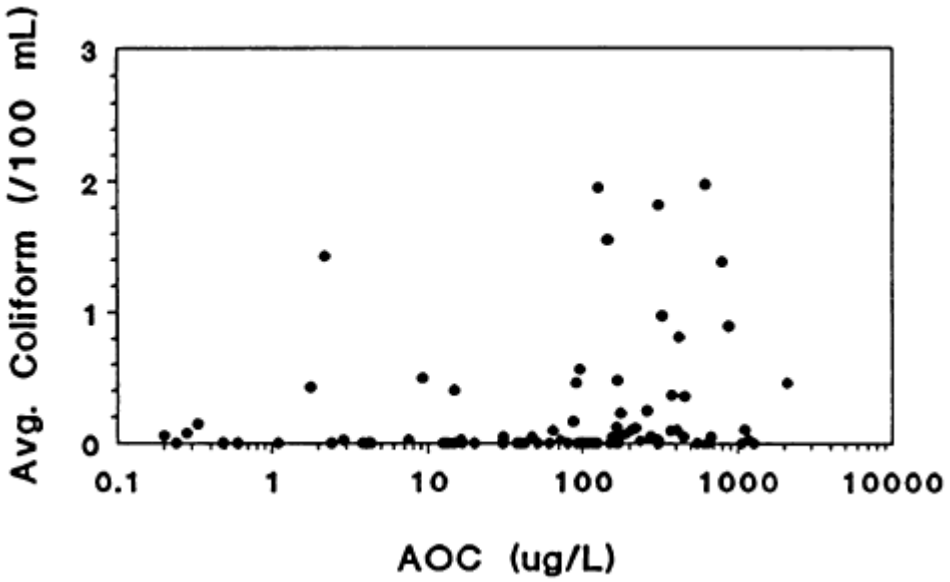

Figure 5. Relationship between AOC levels and coliform bacteria in the DSs seven days later (LeChevalliere *et al*., 1991)

Escobar *et al*. (2001) found that in the DSs fed by ozonated water, HPC were correlated (R^2=0.96) using an exponential model with the AOC. Also it was observed that ozonation caused a significant increase in the AOC concentration of the DS (over 100% increases) as well as a significant increase in the bacterial counts of the DS (average increases over 100%). The HPCs from the DS fed by nanofiltration (NF) in parallel with lime softening water also displayed an exponential correlation (R^2=0.73) with an exponential model based on AOC. No significant correlation was found between bacterial growth on R2A agar and BDOC concentration (Escobar and Randall, 2001). They showed that in full scale operation, NF removed 97% of BDOC from the raw water and suggested that the main mechanism of BDOC removal by NF membranes was size exclusion (Escobar *et al*., 2002). As well with empirical

AOC rejection model developed based on bench scale experimental data they demonstrated a good predictability of the full-scale operating data, suggesting that AOC compounds in the full-scale plant mainly consisted of small molecular compounds with negatively charged functionality (e.g. acetate).

There are several experiments made to set a threshold concentration of BOM below which water can be considered as biologically stable, or water which not promote bacterial regrowth in the DS (Table 1).
These thresholds call on different methodologies for the measurement of BOM concentration and different criteria for the measurement of biological stability. Nevertheless only small discrepancies exist between them, especially in the case of BDOC measurements where values are between 0.15-0.3 mg/L. In case of AOC, this discrepancy is a little higher, with values between 0.01-0.1 mg/L (Prevost *et al*., 2005).

Table 1. Threshold values of BOM for biologically stable drinking water (Prevost *et al*., 2005)

Parameter Followed in the Distribution System	Threshold Values	Measurement Techniques	References
Coliform appearance	<0.05 mg C/L <0.10 mg C/L ≤0.15 mg C/L	AOC AOC BDOCsand	LeChevallier *et al.* 1991 LeChevallier *et al.* 1991 Volk and LeChevallier 2000 Volk and Joret, 1994
HPC increase	<0.01 mg C/L	AOC	Van der Kooij, 1992
No BDOC decrease	≤0.15 mg C/L at 20 °C ≤0.30 mg C/L at 15 °C ≤0.15 mg C/L ≤0.25 mg C/L	BDOCsand BDOCsand BDOCsusp BDOCsusp	Volk, 1994 Volk, 1994 Servais *et al.* 1995 Niquette *et al.* 2001
No BDOC decrease	≤0.15 mg C/L	Modeling approach/ BDOCsusp	Laurent *et al.* 1997

There are appointments to the fact that influence of a biofilm disturbance will be explained with nutrient concentration changes. The findings by Korth *et al*., (2004) shows the results of studies in a network within which treated reservoir water was distributed (Figure 6). From January until May 2003, the HPC (German drinking water regulations methods - GDWR 20°C) in the DS increased significantly from 0 to approx. 80 CFU/mL. Additionally, the nutrient concentration of the finished water increased (determined by laboratory regrowth experiments). From the results it could be deduced that the elevation of the nutrient concentration in the finished water led to a stronger growth on the surfaces and a higher detachment of cells into the water.

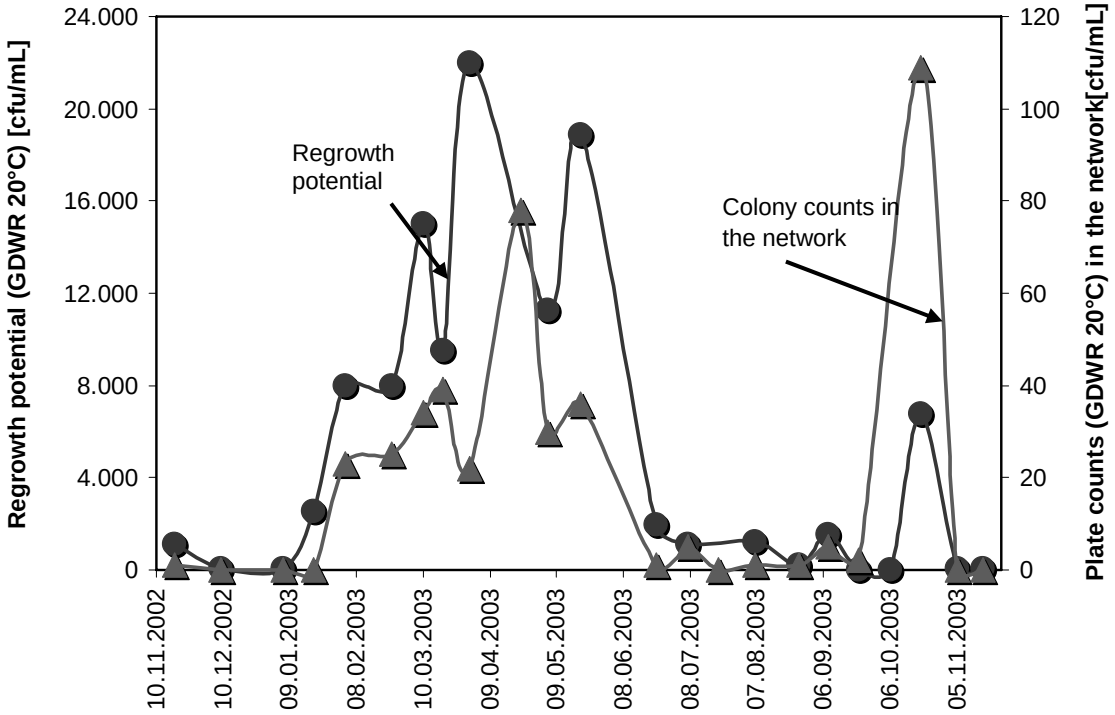

Figure 6. Change of HPC (GDWR 20°C) in a distribution network and regrowth potential for HPC (GDWR 20°C) in the finished water before disinfection (Korth *et al.*, 2004)

This could be confirmed by parallel biofilm studies with biofilm test rigs. As shown in Figure 7, the growth characteristics of HPC (GDWR) in the biofilm and in the bulk water were similar. Changes in the nutrient concentrations of treated reservoir water are often observed (Korth *et al.*, 2004) and are caused by elevated nutrient entries into the raw water layer i.e. by snow melting in connection with spring circulation or mingling with algae-containing water derived from the epilimnion during autumn circulation.

In overall (Escobar and Randall, 2001) concluded that AOC and BDOC provide complementary information and it is advisable, not redundant, to measure both. Measuring only BDOC led to an over-estimation and AOC – to under-estimation of the biological stability.

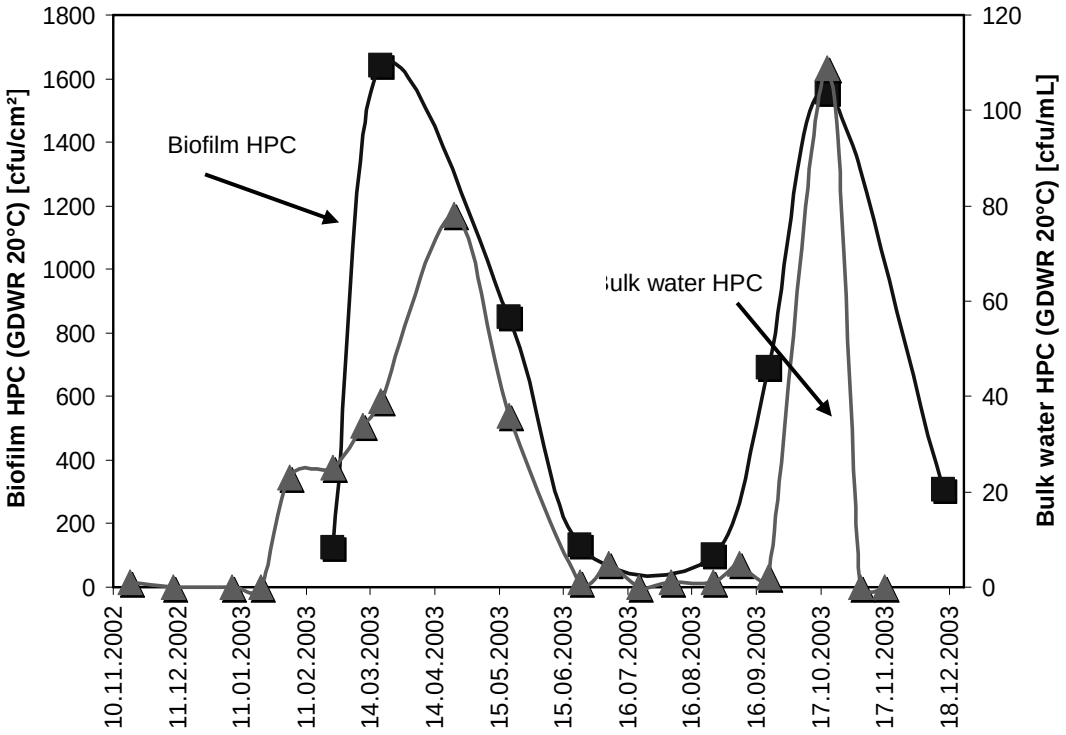

Figure 7. Comparison of the changes of HPC (GDWR 20°C) in the biofilm and in the bulk water of the network (Korth *et al.*, 2004)

1.4.3 Disinfectant

Maintenance of a chlorine residual in the DS is usually recommended to minimize bacterial growth and coliform occurrence. However, as shown by several authors, some European utilities located in the Netherlands, Germany, Switzerland and France are successfully producing and distributing hygienically safe and biologically stable drinking water without a disinfectant residual (Besner *et al.*, 2002). In North America, as well as in the United Kingdom and all around the world, the use of disinfectants remains the favoured approach to control microbiological water quality in the DS (Trussell, 1999). Free chlorine thresholds varying from 0.05 to 0.5 mg/L in full-scale DSs have been quite useful in keeping the rate of occurrence of coliforms low. The only question at this time is whether the disinfectant really inactivates the coliforms or if they are still viable but not-cultivable (VNBC) on selective media (injured coliforms). In a study in a DS in Montreal, Canada, 64% of the positive coliform events occurred at free chlorine concentration under 0.1 mg/L (Figure 8) and stated 20% of events at the high chlorine concentration at one specific sampling point at the upstream of the network which authors explained by intrusion or contamination of a sampling point (Besner *et al.*, 2001). In the study of two Parisian distribution networks, 100% of the positive coliform events occurred for total chlorine concentration under 0.1 mg/L (Volk and Joret, 1994).

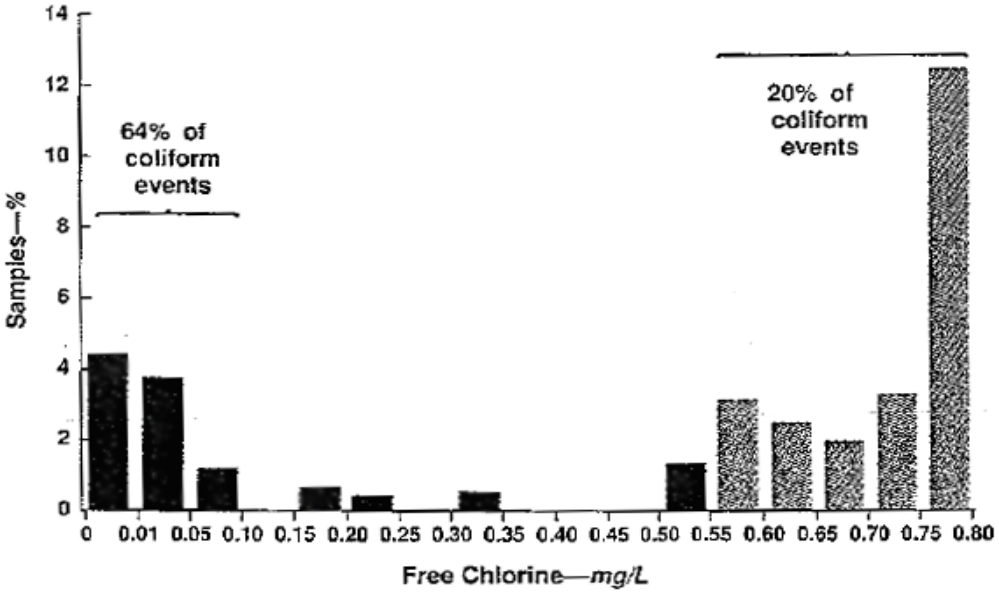

Figure 8. Percentage of samples with positive total coliforms with respect to free chlorine concentration (Besner *et al*., 2001).

Note: Grey bars represent localized coliform events at one specific sampling point at the upstream end of the network

In contrast, there should be mentioned that coliform positive samples have been reported to occur in DSs with free chlorine residual ranging from 0.6 to 12 mg/L. Such chlorine doses may not always be an acceptable solution because of the formation of potentially carcinogenic disinfection by-products (DBPs). Therefore, many utilities chose to use monochloramine as a disinfectant due to these capabilities to persist longer in the DS. However, the use of monochloramine does not necessaryly represent the perfect solution to coliform occurrence in the DS because it is less efficient than free chlorine in controlling a sudden pulse of contamination and can lead to nitrification episodes (Besner *et al*., 2002).

Many studies show a negative correlation between microbial concentrations and disinfectant residual. However, the results of other studies show poor correlation (Woolschlager *et al*., 2001). There should been kept in mind that mentioned negative correlations refers to bulk water bacteria, as chlorine effect on biofilm bacteria and sediments seems to have a more limited effect (Besner *et al*., 2001).

Some of the studies have shown that the bacterial counts in the biofilm rise after a sudden decrease in the chlorine concentration. Shortly thereafter a higher number of bacteria may be released into the water (Korth *et al*., 2007). If there is a continuous fluctuation in the disinfection residual concentrations, a stabilization of the biofilm processes on the surfaces will be inhibited. Thus, a continuous entry of bacteria into the bulk water is possible. As shown in Figure 9, booster chlorination in a front part of the network led to elevated colony counts. In contrast, stable conditions were found in distant parts of the network, where the chlorine residual was lost. At night, when the water

demand was low, the chlorine dosage was not quantity-based but over-proportional.

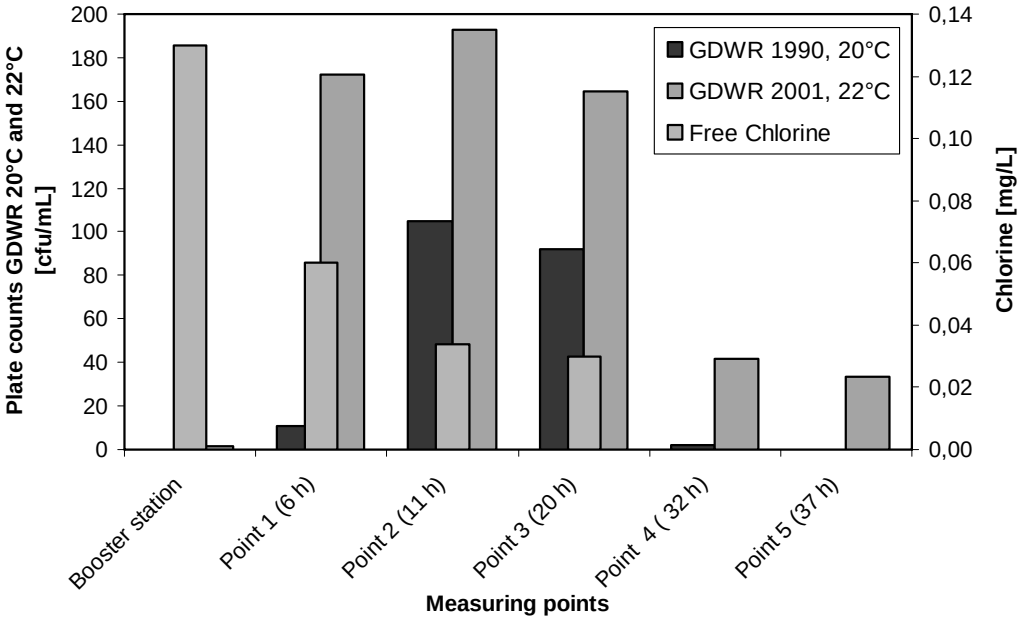

Figure 9. Comparison of heterotrophic plate counts in the network and chlorine residual after a booster chlorination (Korth *et al.*, 2007).

Note: HPC GDWR (1990) 20°C, HPC GDWR (2001) 22°C; numbers in brackets (X-axis) show the average water age at the analogous measuring points; the distinct points were not placed on the same pipeline but were chosen to be spread over the network

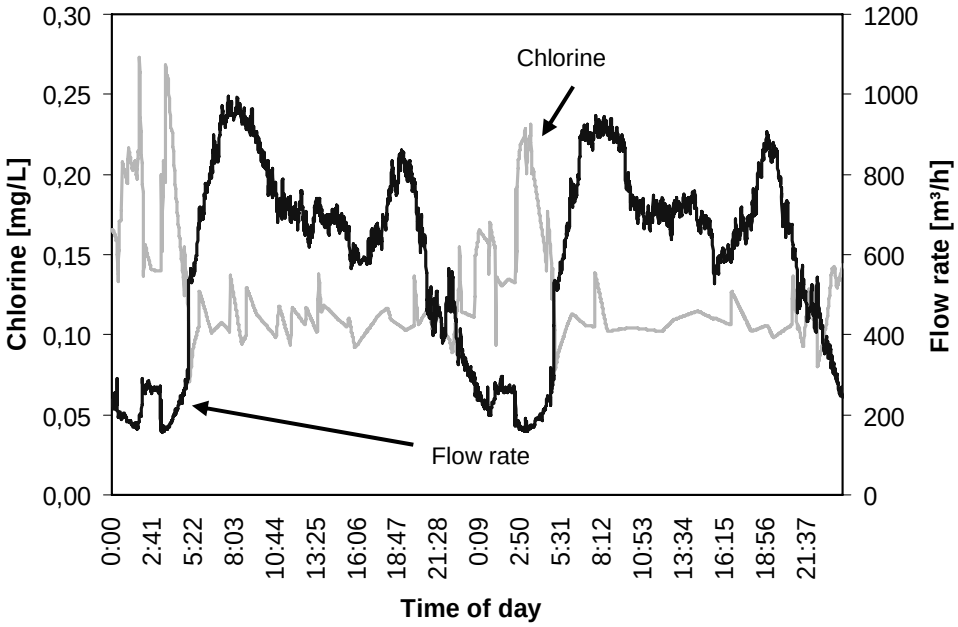

Figure 10. Fluctuations of chlorine concentration (green) in the outlet of a booster station (Korth *et al.*, 2007)

Addendum to Fig. 9.

Therefore, the chlorine concentration in the outlet rose significantly (Figure 10). With increasing water demand during the morning hours, a water portion with a higher chlorine concentration was sent through the front part of the network for a short time. This way, the biofilm constitution was influenced so that more bacteria were released into the water afterwards due to a stronger growth on the surfaces.

1.4.4 Velocity

The velocity of water flowing through the mains is an important factor influencing microbial colonization and may regulate growth in several ways in the DS. Pipe sections with high water velocities tend to limit microbial protections and reduce sediment accumulation, thus minimizing nutrient entrapment and protection from exposure to the disinfectant, but on the other hand increased velocities allow for greater flux of nutrients. Conversely, areas of slow flow and dead-end locations have been statistically correlated with water quality deterioration because of loss of disinfectant residual (Rice *et al*., 1991) and that results in high bacterial counts at the customers tap (Brazos *et al*., 1982; LeChevallier *et al*., 1987). Reversal of water flows can shear biofilms and the hammer effect can dislodge tubercles from pipe surfaces. Opeim *et al*., (1988) found that bacterial levels in an experimental pipe system increased 10-fold when flows were started and stopped. Larger releases of bacteria were noted when the pipe system was exposed to physical and vibration forces.

The recent findings by German researchers showed that the susceptibility of a biofilm, i.e. to what extent bacteria are released into the water, depends on the biofilm constitution. As shown in the research results by Korth and Wricke (2004a), no significant changes in the bacteriological parameters were observed during a three-week-stagnation in previously well flowed pipes, in which a well developed biofilm was to be assumed according to the preconditions. Therefore, stable microbiological conditions existed in these pipelines. In contradiction to these results, a significant regrowth was recognizable in a laboratory regrowth experiment with the same water in glass bottles. Figure 11 shows a representative example of these studies. The main difference between the stagnation in the pipeline and the regrowth experiment in the bottle was the development of the biofilm. There was no elevated bacterial release in the pipe with a well developed biofilm. In contrast, a three orders of magnitude higher bacterial regrowth was found in the bottle due to growth on the surface (without biofilm) and release of bacteria into the water.

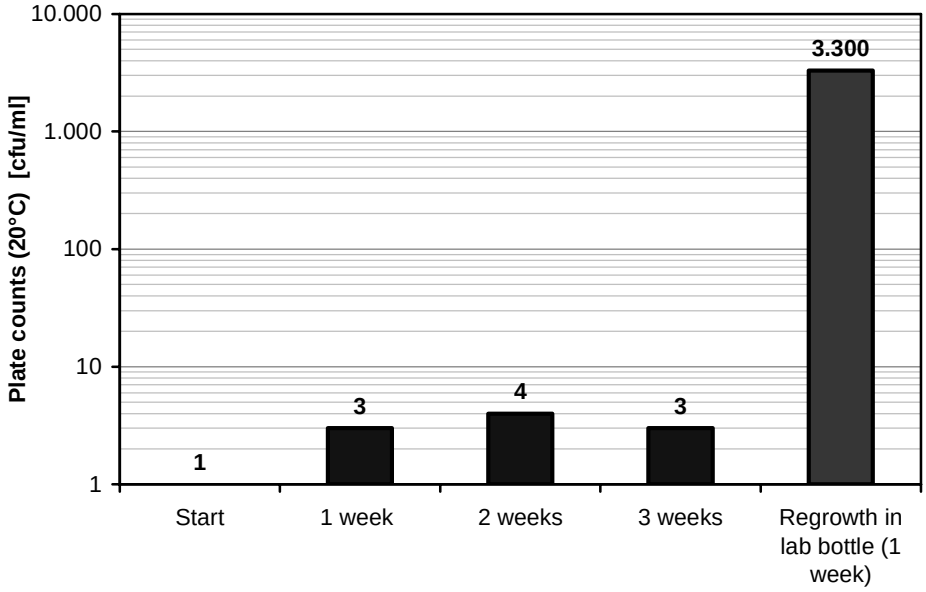

Figure 11. Comparison of HPC (GDWR 20°C) changes during stagnation in a recently well flowed distribution pipeline and as a laboratory regrowth experiment in glass bottles Korth and Wricke (2004a).

Note: Surface-area to volume ratio pipeline: 1:50, surface-area to volume ratio glass bottle: 1:68

The same effects as in the laboratory regrowth experiment were observed in stagnation experiments in slowly or rarely flowed end parts of the network. Because of the lower nutrient concentration in these pipes, the biofilm was suggested to be less developed than in well flowed pipes. During stagnation, colony counts in these pipes increased much more than in well flowed pipes. Figure 12 shows two representative examples of a continuously flowed (Pipe 1) and a discontinuously flowed pipe (Pipe 2). Data shown are the maximum HPC on R2A-plates during a three-week-stagnation. Hence it was assumed that also in less flowed pipes with little developed biofilm bacteria are increasingly released into the water phase during growth on the surfaces. A stabilization effect was observed in these pipes due to a regular change in the water volume.

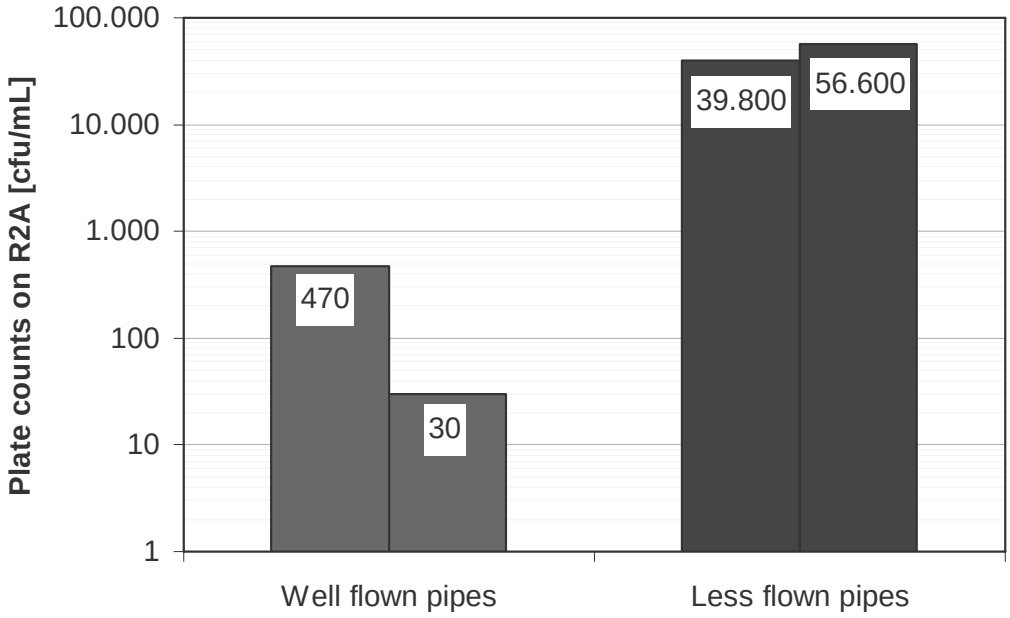

Figure 12. HPC (R2A) maxima during stagnation in a well flown pipe (TOC: 2,5 mg/L, AOC: 6 µg/L) and a less flown pipe (TOC: 1,0 mg/L, AOC: 7 µg/L; two representative examples) Korth and Wricke (2004a)

As shown in Figure 13, the growth factor for TBN (maximum divided by initial value) decreased analogous to the number of experiments. Thus it appears that the periodical change in the water volume in the previously scarcely flowed pipe led to a gradual improvement in the biofilm conditions due to the improved nutrient supply so that bacteria were released into the bulk water on a small scale during growth on the pipe surfaces.

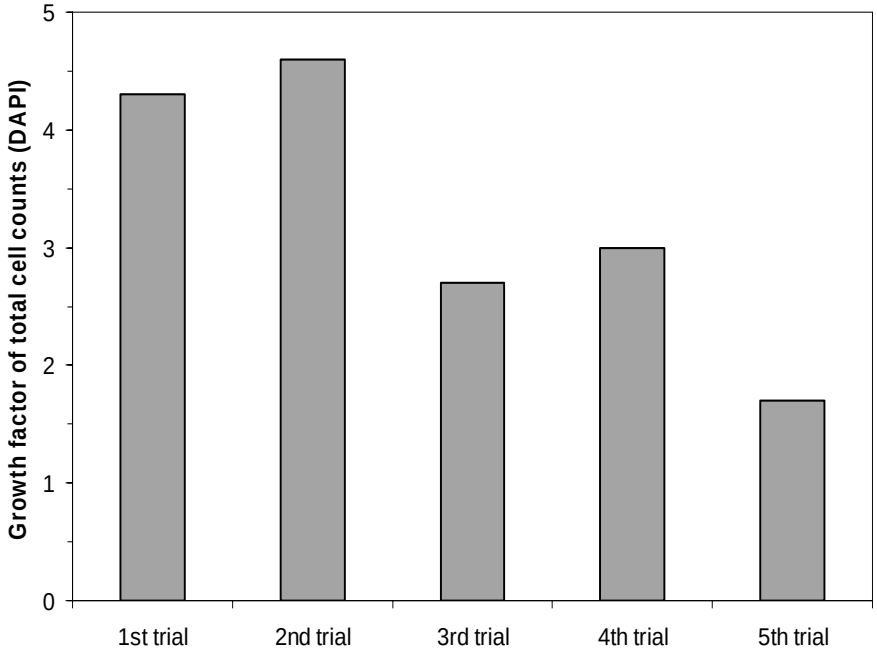

Figure 13. Decrease of growth factor of total cell counts (DAPI) in line with the number of stagnation experiments in a previously scarcely flown pipe Korth and Wricke (2004a)

Lehtola *et al*., (2006) found that the increase of water flow velocity from 0.03-0.04 m/s to 0.19-0.28 m/s increased growth of biofilm measured as TBN and HPC and caused an immediate increase in bacterial number in bulk water as a result of detachment. These findings were made in lab-scale conditions with 10 mm copper and 12 mm PE pipes.

The influence of biofilm development on regrowth during stagnation in supply pipes could recently be confirmed in test rig experiments conducted at TZW Dresden (Korth *et al*. , 2007).
As can be seen in Figure 14, the amount of regrowth decreased with increasing operation time. After 182 days of continuous operation, regrowth (HPC, GDWR) was less than one order of magnitude within six days of stagnation. However, biofilm TBN and HPC (R2A), respectively, reached steady state within the first 70 days of operation, approximately (data not shown), while biofilm HPC (GDWR) showed greater variance within the first 182 days.

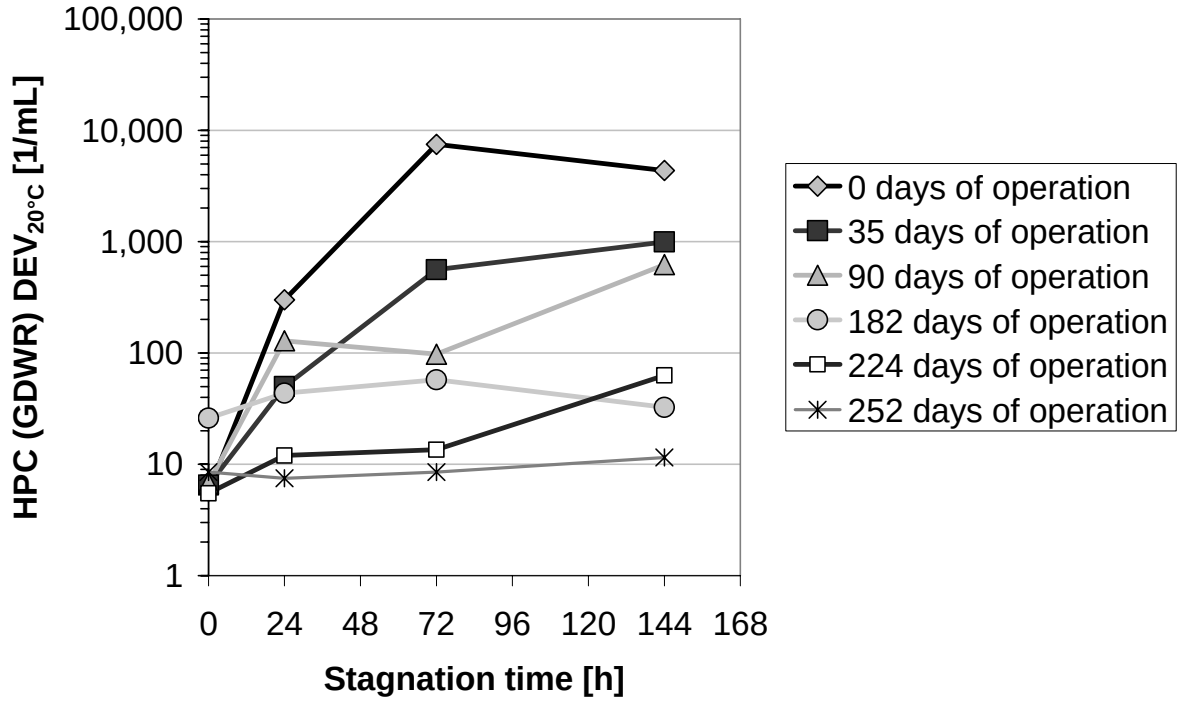

Figure 14. Stagnation experiments in test rig pipes operated for 252 days under continuous operation (0,1 m/s) including 6 interruptions with 6 days of stagnation, respectively (Korth *et al*. , 2007)

These findings are in agreement with Dutch and British findings in drinking water DS hydraulic field. Vreeburg and Boxall (2007) proposed that sediments will tend to accumulate in areas with low velocities, such as dead ends, over sized pipes and redundant loops. Such features are common in most networks as the systems are designed to comply with large fire fighting demands that are typically far greater than consumer demands, particularly

at the extremities of large systems and in smaller systems. The same opinion about oversized drinking water DS networks proposed Okun (2006). The approach is based on a fundamental rethinking of the fire fighting demands. The new design philosophy results in a distribution network that is branched with pipes of relatively small diameter. These lead to higher velocities within the systems and can reduce discolouration problem proposed by Dutch researchers and as well fits for findings by German researchers mentioned above for decreases of bacteria releases in bulk water from biofilm.
The new design rules are widely applied in the Netherlands resulting in a reduction in the average pipe diameter by length in new networks, the rules are not only accepted on the basis of improved water quality but also because the new networks are on average 20% cheaper to construct than conventional ones. The savings are mainly achieved by the reduction in the length of the pipes because the loops are not closed anymore (Vreeburg and Boxall, 2007). Okun (2006) proposed split networks one for drinking water and another for fire fighting. As well water supplied for fire fighting can be left untreated therefore it is possible to reduce the amount of water which should be treated.

1.4.5 Substratum and sediments

The intensity of bacterial colonization on DS pipes surface is influenced by pipe material characteristics. The presence of iron tubercles and corrosion processes has also been associated with microbial growth, biofilm formation and especially with colonization by coliform bacteria. This is due to fact that chlorine is depleted in reaction with pipe material and sediments. A positive relationship has been found to exist between the number of miles of unlined cast-iron pipes in filtered, free-chlorinated DSs and coliform occurrences in the study of LeChevallier *et al*., (1996), suggesting that the corrosion of iron pipe surfaces is an important factor affecting coliform occurrences (Besner *et al*., 2002). In the studies by LeChevallier *et al*., (1990) there was found that a level (1 mg/L) of either free chlorine or monochloramine could reduce viable counts by greater than 100-fold (2 logs) for biofilm grown on galvanized, copper, or PVC pipe surfaces. However, when the microorganisms were grown on iron pipes, free chlorine residuals ranging from 3 to 4 mg/L were ineffective for biofilm control. In this situation, only monochloramine residuals above 2 mg/L were successful for reducing biofilm viable counts which can be explained by better penetration of monochloramine capabilities into the biofilm layer and inactivation of bacteria. As well Lehtola *et al*., (2005) expanded these studies and found that biofilm formation in chlorinated waters is more favourable on PVC pipes than on copper pipes.
Lehtola *et al*., (2004) found that new plastic pipes released some phosphorus, which in regions where phosphorus is limiting for bacterial growth in water, lead to higher TBN in biofilms.
In order to decrease corrosion and thereafter lower levels of TBN within biofilms in some countries are produced special actions. Common mechanisms of corrosion control include increasing pH, remineralization, and the addition of phosphates or silicates (Besner *et al*, 2002).
Regarding to particulate mater which they are associated with higher bacteria number in biofilm in a DS may originate from various sources, including

incomplete removal of particles from raw water, release of fines from filters, precipitation of metal oxides or calcium carbonate, external contamination in pipes and reservoirs, postflocculation, biological growth, and corrosion. To remove sediments from water pipes, flushing programs may be undertaken and it may be advisable to choose unidirectional flushing. There should be noticed, that increased chlorination and flushing of the DS in New Haven, USA, actually increased coliform levels in drinking water, what can be explained by detachment of biofilm whereas coliforms proliferate (LeChevallier, 1990). It was reported that three days after systematic flushing of the DS in Muncie, USA, 126 coliforms/100 mL were recovered just a few blocks away from the flushing area.
As well Ridgway and Olson (1982) reported that most of the chlorine-resistant microorganisms detected in a drinking water DS were associated with particles. LeChevallie *et al*., (1981) suggested that high turbidity may play a role in coliform survival in the presence of chlorine residual because coliforms associated with particles may be protected from contact with the disinfectant.

1.4.6 Pipe age

Nagy and Olson (1985) observed a correlation between the years in service of a pipeline and the bacterial density. They estimated that HPC bacterial levels increased one log for every 10 years of service.

1.4.7 Rainfalls

Rainfall has been suggested by some investigators to be a catalyst for coliform growth. Lowther and Moser (1984) found that TOC levels in raw water were at their highest when turbidity increased after rainfall. LeChevallier *et al*., (1991) observed a seven-day lag between rainfall events and the occurrence of coliform bacteria in DS water samples (Figure 15). The authors speculated that rainfall washed nutrients into the watershed and, after a transit period and growth lag, resulted in increased bacterial densities.

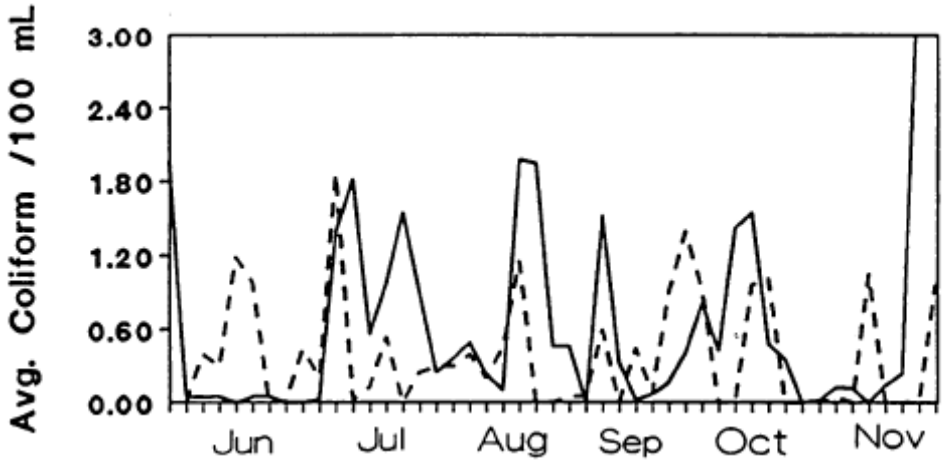

Figure 15. Relationship between rainfall (dashed line) and daily coliform level (solid line). Coliform data have been offset by 7 days (LeChevallier *et al*., 1991)

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2 CONCEPTUAL MODELS FOR BACTERIAL REGROWTH IN DRINKING WATER NETWORKS

2.1 State of art for regrowth models

Bacterial regrowth causes water quality deterioration in the water distribution networks including: appearance of taste and malodor, turbidity, loss of residual chlorine and increased risk of pathogens survival. The interaction between different factors in the networks is very complex, thus, prediction of regrowth based on earlier observations (statistical multiple regression analyses) has not been entirely successful.

The biological, chemical, and physical processes in any ecosystem are represented in the model by means of mathematical equations that are the relations between forcing functions and state variables and may contain some coefficients and universal constants (Williams and Jorgensen, 1989). A mathematical model is a systematic attempt to translate the conceptual understanding of a real-world system in to the mathematical terms. A model is a valuable tool for testing our understanding of how system works and with model we can regulate and/or manipulate with real-world processes. The golden rule of modeling is that a model should be as simple as possible, and only as complex as needed.

Although the use of the mathematical models for hydraulic analysis of DSs dates back to the 1930s (Cross, 1936), water quality models of DSs are a relatively recent development. Wood (1980) represents a steady-state hydraulic model of slurry flow in distribution network where a series of simultaneous equations are solved for each node. Similar models were used later where simulation of hardness by Chun and Selznick (1985) and study of blending, settling and flushing in DSs by Metzger (1985). Males *et al*. (1985) develop model SOLVER which used simultaneous equations to calculate the spatial distribution of concentration and travel times associated with DS links. Models that simulate the trace contaminants and water age in DS under temporally varying conditions were reported by Grayman *et al* (1998) and Kroon and Hunt (1989). More recent developments include the effects of both bulk and wall reactions in simulations of chlorine residual (Rossman *et al*. 1994; Vasconcelos *et al*. 1997).

Bacterial regrowth models distinguish three compartments, such as the substratum onto which the biofilm accumulates, like pipe walls; the biofilm itself and the overlying bulk water. Some models predict that regrowth process occur only in biofilm. Each compartment contains characteristic dissolved components, such as substrates, and particulate components, such as bacteria. The components are consumed or produced through transformation processes, and they move about the biofilm space through

transport and transfer processes. All the processes that affect a component in a particular compartment must be summed up through a mass balance.

Over the last decade several mechanistic models for biofilm growth including model by Camper *et al*. (1994), Lu *et al*. (1995), SANCHO (Servais *et al* 1995), PICCOBIO (Dukan *et al.* 1996, Piriou *et al*. 1998), BAM (Camper *et al*. 1996) and model by Bois *et al*. (1997) have been developed. More recent studies give a model of live and dead cells by Jagatheesan *et al*. (2000, 2004), models by Huck and Gagnon (2004) and by Zhang *et al*. (2004). Most of these mechanistic models of regrowth have been adopted from well-known concepts in biofilm modeling of water and wastewater treatment processes.

Although the some models have been used in the full-scale with various degree of success (Laurent *et al.* 2005, Piriou *et al.* 1997) important shortcomings including poor relation to hydraulics of distribution networks and their unjustified complexity in describing biological phenomena (Zhang *et al.* 2004) are probably restraining them for wider application by water industry. The models are designed for idealistic systems and do not take pipe corrosion and sediment accumulation in the DSs into account. Besides, the fact that other substrate than carbon can control biofilm regrowth is not considered in these models. The further improvement of commercial biofilm regrowth models such as SANCHO, PICCOBIO are limited because they are proprietary. Overview of the some above mentioned biofilm growth models are given further in this chapter and summarized in Appendix.

The conceptual model in which many of the problems (Zhang *et al.* 2004) are mitigated will be used as a base for mathematic regrowth models development in TECHEAU project. The model describes the following microbial processes: free and attached growth, detachment, endogenous respiration, and inactivation by chorine. Suspended and attached bacterial number, decrease of chlorine residuals and changes of biologically degradable carbon in time could be calculated using this model. Our analysis has shown that the models should be upgraded further. The decision to include important variable such as cast pipe corrosion, suspended particle transport, type of pipe material, and hydraulic fluctuation within the model will be made later during this project.

2.2 SANCHO model

SANCHO model was adapted from the model of biological filtration on GAC (CHABROL model) developed by Billen et. al (1992), while authors consider that there are an analogy between the processes occurring during progression on the water within a long pipe with attached bacteria on its wall, and those occurring during filtration through a solid support. The processes taken into account in the SANCHO model proposed by Servais (1995) are the following: enzymatic hydrolysis of dissolved organic matter by bacteria and growth of free and fixed bacteria on the hydrolysis products; bacterial mortality which releases organic matter; reversible adsorption of bacteria and their biological

attachment to inner pipe surface; chemical consumption of free chlorine and impact of free chlorine on the activity of free and fixed bacteria (Figure 16.).

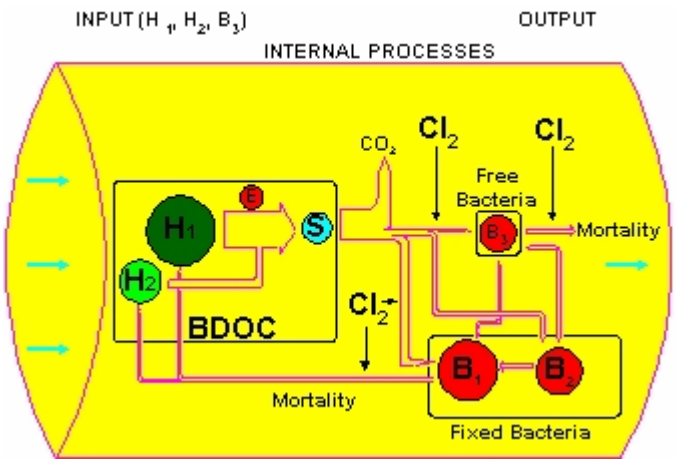

Figure 16. Schematic representation of SANCHO model (Laurent *et al*., 1997).
Legends: three types of BDOC (H1-rapidly hydrolysable polymeric BDOC, H2-slowly and S-directly assimilable substrate); B1-biologically fixed bacteria, B2-adsorbed bacteria, B3-free bacteria

Organic matter was utilized by bacteria according to Michaelis-Menten kinetic. Bacterial mortality is considered to obey first-order kinetics with regard to bacterial biomass, it is characterized by the first order constant k_d ranging from 0.03 h^{-1} (Servais *et al*., 1995) to 0.06 h^{-1} (Laurent *et al*., 1997). For attachment of bacteria on a solid support authors considered two different processes: a rapid and reversible physiochemical process; and a slow irreversible biological attachment process involving active bacterial secretion of polysaccharides. They used Langmuir's theory for describing the kinetics of the adsorption/desorption process. It assumes that the rate of bacterial adsorption onto the support is proportional to the concentration of the bacteria in the bulk phase and to the concentration of the free adsorption sites on the support. Desorption is assumed to be a first-order reaction with respect to the concentration of attached bacteria. For SANCHO model initial adsorption rate and concentration of adsorbed and suspended bacteria at the equilibrium were experimentally determined on cast iron. Chlorine depletion dynamic is assumed to be follows first-order kinetics with regards to chlorine concentration and total chlorine demand. They assumed that in water phase the total chlorine demand is due to DOC in range 1 to 3 mg Cl_2/mg DOC. The impact of chlorine on the activity of bacteria was determined experimentally. The difference in sensitivity of attached and suspended bacteria was very small, while some authors show that attached bacteria are more resistant to the free chlorine action.

To calculate SANCHO model seven variables must be specified. Model calculates the spatial gradients, at steady states, of BDOC and chlorine concentration, free and fixed biomass. The model considers the case a water mass flowing during given residence times (up to 20 hours) in successive pipes of decreasing diameters (1250 – 100 mm). In order to validate the

SANCHO model, experimental data collected from two DSs in Paris were compared with model calculations. Comparison showed good correlation for disinfectant depletion (correlation coefficient 0.92), BDOC (0.74) and suspended bacteria (0.66). For attached bacteria statistical analysis was not performed because experimental data was insufficient.

2.3 PICCOBIO model

PICCOBIO model developed by Dukan *et al.* (1996) combines a hydraulic model (PICCOLO) with a water quality model, including BDOC, chlorine residual and bacteria. From this model, a BDOC value of 0.25 mg/L and a temperature of 16 °C were derived as threshold values above which problems can be expected. These values are in agreement with observations in practice. Model is constructed by using hydraulic results previously generated by PICCOLO and a numerical scheme to predict bacterial count at each node and on each link of a network and to locate the zones where the risks of biological proliferation are the highest. Model uses the graphic interface of PICCOLO and provides an effective and easy way to visualise on a computer screen water quality variations in the network, using a colour code for bacterial count, nutrient concentration and chlorine residual.

PICCOBIO model takes into account the growth of suspended and fixed bacteria, the consumption of available nutrients in the bulk water and in the biofilm layer, the influence of chlorine residual on the mortality of suspended and fixed biomass, as well the natural mortality of bacteria by senescence and grazing; the deposition of suspended bacteria and the detachment of biofilm cells, the influence of temperature on bacterial activity and chlorine decay; as well the chlorine decay kinetics under the influence of hydraulics and pipe materials (Figure 17).

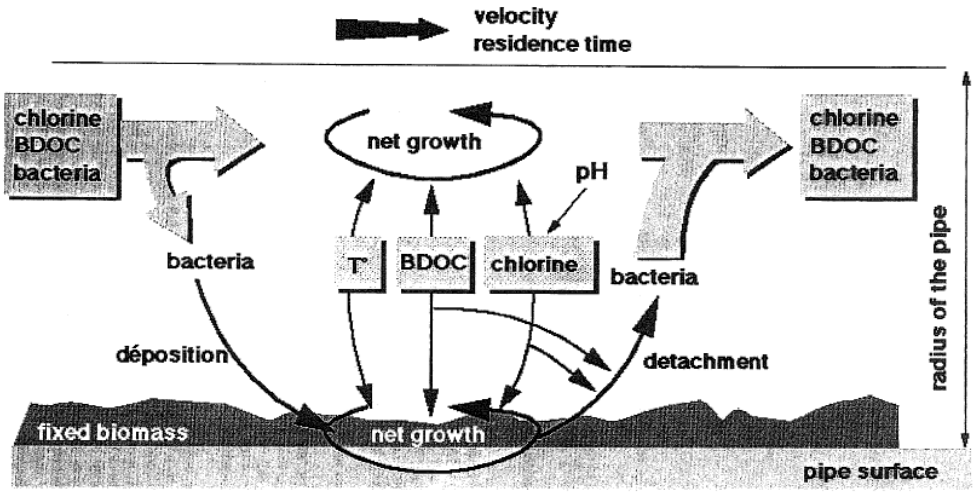

Figure 17. Phenomena taking into account by the PICCOBIO model (Dukan *et al.*, 1996)

The modelling of the fixed biomass as a layer uniformly distributed over the pipe surface, expressed as an equivalent thickness of carbon, has been adopted. By this way, a differentiation between the mathematical expression of the free and that of the fixed biomass was made in the model. This mean it

is possible to distinguish between phenomena depending on their locations: reactions in solution, reaction at the water/biofilm surface interface and within the biofilm.

PICCOBIO model proposes also an original approach for chlorine bactericidal action on suspended and fixed biomass. To model the action of chlorine on the fixed biomass and its stronger resistance compared with the free biomass, the diffusion of the chlorine through the boundary layer and the biofilm has been taken into account. This calculation of the average penetration depth of the chlorine front into the biofilm enables the identification of two layers: a chlorinated layer and a layer not attained by the chlorine which provides a material indication of the better resistance of the fixed biomass. As detachment is a key phenomenon in the modelling of bacterial dynamics in DSs, the influence of different formulas of detachment kinetics on the mathematical expression of model variables were determined by solving model equations.

The first model calibration was done using data obtained from a pipe loop system pilot under various operating conditions as a perfectly mixed reactor. Model calibration indicated that difference between simulated and measured data for total and viable bacteria were less than 10%. The model has been also used to simulate a variety of DSs of different sizes and levels of details and a validation of the model has been carried out by means of measurement campaigns on different DSs. Results obtained from model calibration for two champagne with data from Marseille distribution network showed good relationship between simulated and measured viable bacteria count (r^2 = 0.795, n=15) confirms the accuracy of the model.

2.4 BAM model

The Biofilm Accumulation Model (BAM) was developed by the Center for Biofilm Engineering at Montana State University (Camper, *et al*., 1996). BAM simulates the evolution of a mixed-culture biofilm system within a series of completely mixed units. It is a modified version of a traditional biofilm modelling program (BIOSIM) developed at the Swiss Federal Institute for Water Resources and Water Pollution for application to water and wastewater treatment processes. This model has not yet been linked with a hydraulic model.

2.5 Model by ZHANG *et al.*

To develop a mathematical model of bacterial regrowth Zhang *et al*., (2004) combined hydraulic network calculations, inclusive of unsteady-state flow conditions and dispersion, with a description of free and attached bacteria growth, detachment, endogenous respiration, and inactivation by chlorine. Zhang *et al*., (2004) suggest simplification in to the description of the bacterial growth, attachment, detachment and inactivation given in SANCHO or PICCOBIO. They consider that the processes in the biofilm system are not quite understood. Therefore overly detailed descriptions about those processes are not reasonable.

A statistical sensitivity analysis (Cigana *et al*., 1997) showed that only five factors (bacterial mortality, maximum capacity for bacterial fixation, bacterial yield, initial substrate concentration, and maximum uptake rate of the substrate) strongly influence prediction of free bacteria in the bulk water. Zhang consider that the same analysis with the field data should be performed to determine the most significant factors that influence the biofilm growth. Moreover the non-important processes should be reduced and combined in current existing models. Zhang suggest that the detachment of the biofilm into water due to hydraulic shearing is the most important factor to release bacteria into bulk water to cause the bacterial regrowth in water DSs.

The model developed by Zhang contains similar microbial and chemical processes descriptions used in other water quality model. The following major assumptions governed the development of this model: l. advective - dispersive transport occurs in the axial direction of the pipe but not in the radial direction; 2. the hydraulic dispersion coefficient is the same throughout the pipe network, although a coefficient that varies with velocity and pipe diameter could be easily included in the future simulations (molecular diffusion is lumped into hydraulic dispersion); 3. the dependent variables are free bacteria in the bulk water, attached bacteria (biofilm) on the inner surface of the pipe wall, biodegradable dissolved organic carbon (substrate) in the bulk water, and chlorine in the bulk water (other disinfectant could be substituted); 4. transformation exists between free and attached bacteria, i.e., free bacteria can deposit as attached bacteria and attached bacteria can detach to form free bacteria; 5. attached biofilm is a uniform thin layer of biomass such that diffusion of substrate and chlorine into the biofilm is not rate limiting (Huck and Gagnona 2001); 6. the bacterial growth rate is only controlled by biodegradable organic carbon concentration, temperature and chlorine concentration (inhibition function).

The Monod formulation is used to account for the dependence of substrate concentration. The empirical inhibition relationship of chlorine on bacterial growth was used by Laurent *et al*. (1997) in the SANCHO model but the empirical approach to modelling the effect of temperature – by Dukan *et al*. (1996) in the PICCOBIO model.

To develop a dynamic biofilm growth model they couple the proposed simplified, modified model with a robust hydraulic model like EPANET. To simplify the construction of a dynamic model linking bacterial processes with the hydraulic description of the network they use a hypothetical network under steady-state conditions similar that of Cigana *et al*. (1997) to determine if entire sub components of the model could be eliminated.

2.6 Model by LU *et al.*

Lu *et al*. (1995) developed a biofilm model that accounts for simultaneous transport of substrates, disinfectants, and microorganisms and that predicts

substantial changes in quality of distributed water. The model consists of a set of mass balance equations for organic substances, ammonia nitrogen, oxidized nitrogen, dissolved oxygen, alkalinity, biomass, and disinfectants in the bulk liquid phase and within the biofilm under laminar and turbulent flow conditions. This model is validated by comparing its solutions with numerical solutions in the literature and is then applied to predict the behaviour of a typical water treatment plant effluent through a distribution pipe. The flow properties and disinfectant consumption rate at the pipe wall play a significant role in the determination of drinking water quality in the DS, and the chemical oxygen demand is used as the growth substrate parameter.

2.7 Shortages and suggested improvements of common bacterial regrowth models

SANCHO and PICCOBIO are two most widely used models for simulation of bacterial regrowth in water distribution networks. SANCHO suffers two major drawbacks. First, the process descriptions of growth, attachment, detachment and inactivation of bacteria are not linked with a hydraulic model. Instead, a hydraulic model must be first run to determine the residence time at each location of interest under some steady state condition of flows and these results are then used to run SANCHO. As a result, the model does not provide a dynamic prediction of bacterial regrowth as would be true due to variations in water velocity and water quality changes entering the DS in time. Second, a total of 19 input parameters must be specified. A statistical sensitivity analysis of SANCHO has recently been completed (Cigana, *et al*., 1997). Of the 19 input parameters, five (bacterial mortality; maximum capacity for bacterial fixation; bacterial yield; initial substrate concentration; and maximum uptake rate of substrate) have been shown to strongly influence prediction of free bacteria in the bulk water.
The complications in SANCHO arise from overly detailed modelling of substrate and attached growth. Substrate is divided into rapidly and slowly hydrolyzed polymeric BDOC which then produce monomeric material for bacterial uptake. Six rate constants must be specified when only two may be necessary if the hydrolysis reactions are not included. There are also two parameters to allow dead bacterial biomass to be converted back to these rapidly and slowly hydrolyzed polymeric BDOC fractions; the importance of this material on continued bacterial growth needs to be examined within the model. The subdivision of attached bacteria into fixed and adsorbed may also be worth investigation. The SANCHO model does not include the effect of water velocity on bacterial shear, chlorine demand exerted by pipe wall material nor differences in attachment potential of bacteria to different pipe materials. Neither models takes into account that in some water supply systems other nutrients than carbon can become bacterial growth limiting.

PICCOBIO offers the advantage over SANCHO of coupling with the hydraulic network model (Dukan *et al*., 1996). This is a dynamic model; hence it describes the concentration of any species as a function of time and position within the DS. The mathematical descriptions of bacterial growth, attachment, detachment and inactivation by disinfectant are much more

complicated than in SANCHO, which increases the number of parameters to be specified.

Unlike biofilm models in treatment processes, prediction of the release of bacteria from the pipe surface is more important than the biodegradation of substrate. Thus, the description of bacterial shearing is especially critical (Stewart, 1993). The detachment rate of bacteria depends on description of shear and its relation to water velocity in the pipe. This is included in PICCOBIO but not in SANCHO. The developers of PICCOBIO acknowledge the need for better understanding of shear. For instance, the tendency to shear bacteria could also be related to the stage of bacterial growth stage (fast, slow or endogenous decay) as was found by Speitel and DiGiano (1987) in analysis of bacterial release from granular activated carbon beds.

Finally, both SANCHO and PICCOBIO are proprietary models. Thus, only a few investigators have published papers describing the model formulations, sensitivity analysis and calibration with field data. There is a need for involvement of more researchers in critique of these models, however this is not possible because complete scientific information about these models not available.

The model developed in TECHNEAU will address most important issues described above but at this stage of model development the following specific improvements are suggested:

- the coefficients of yield, which in this mathematical model are same as for suspended and attached bacteria. Higher yield for suspended bacteria (more bacteria produced from the same amount of carbon) than for biofilm bacteria will be assumed in the model. As well additional coefficients of yield for new equation system of phosphorus balance (see further suggestion).
- The maximum growth rate different for suspended and attached bacteria.
- Model should contain two possible scenarios for bacterial regrowth. According to the nutrients ratio in bulk water growth rate-limiting substrate could be either carbon or phosphorus. Optimal ratio of carbon, nitrogen and phosphorus is about 100:10:1. Therefore equation system of growth rates should contain option of phosphorus influence and there should be additional equation for phosphorus utilization.
- New mass balance equation for phosphorus utilization should contain option for phosphorus recirculation. Dissolved phosphorus regeneration in range of 210-885 pM h^{-1} is well established in limonological environments (Hudson *et al.*, 2000), thus models allow to test this phenomena in water supply systems. And additional coefficient of phosphorus release from pipes should be tested.
- Wall reaction of chlorine due to pipe material. Cast iron pipe corrosion should be updated.
- Threshold values for chlorine influence on suspended and attached bacterial activity $Cl_{2,t}$.

- Suspended particle accumulation and transport in the DSs should be included.
- Bacteria release from pipe surface should be linked with water quality, concentration of chlorine and temperature disturbances.
- The model should be complement with dynamic conditions in time.

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2.9 Appendix

Table 2. Comparison of Biofilm-Distribution System Models

Model	Linked with hydraulic model?	Constants	Input parameters	Output parameters	Growth limiting nutrient	Model calibration and validation?	Proprietary
SANCHO	No	19, obtained experime-ntally	Temperature, chlorine, BDOC classified in three classes of biodegradabil ity, free bacteria	Fixed and free bacteria measured as ug C/cm2, chlorine profile, BDOC profile, reduction thresholds for BDOC	Carbon	Field from two French full-scale DSs and latter from four Canadian and three French	Yes
PICCOBIO	Yes, PICCOLO	obtained from literature	Temperature, chlorine, nutrients	Fixed and free bacteria, high risk zone	Carbon	Pilot pipe loop system and data from two Marseil, France DSs	Yes
BAM	No	???	AOC	HPC bacteria and coliforms, AOC, biofilm thickness	Carbon	-	???
ZHANG	Yes	12, obtained from literature, although some are corrected	Temperatu re, chlorine, BDOC, free bacteria, biofilm bacteria	Chlorine, BDOC, free bacteria, biofilm bacteria	Carbon	Data from literature, required experiments	No

3 RIGA MODEL

3.1 Conceptual description of RIGA model

Biofilm regrowth in RIGA model is modelled using the main assumptions and equations proposed by Zhang *et al.* (2004) for straight sufficiently long pipes. Within the first 15 months of the project there was written the model for long pipe and model for biofilm reactor Propella TM using exactly same equations (see above). The model for Propella TM is useful to verify existing model constants in lab-scale conditions. For both models the code uses a subroutine from IMSL library (see figure 18) with exception for biofilm reactor Propella TM which water quality variables are renewed every 15 hours. However, this is a water detention time in the reactor and can be changed. This means that the water quality is renewed within a constant cycle of 15 hours, but bacteria concentration in the biofilm is accumulated. Simulations for comparability of results for both types of models have been made: pipe and Propella TM reactor.

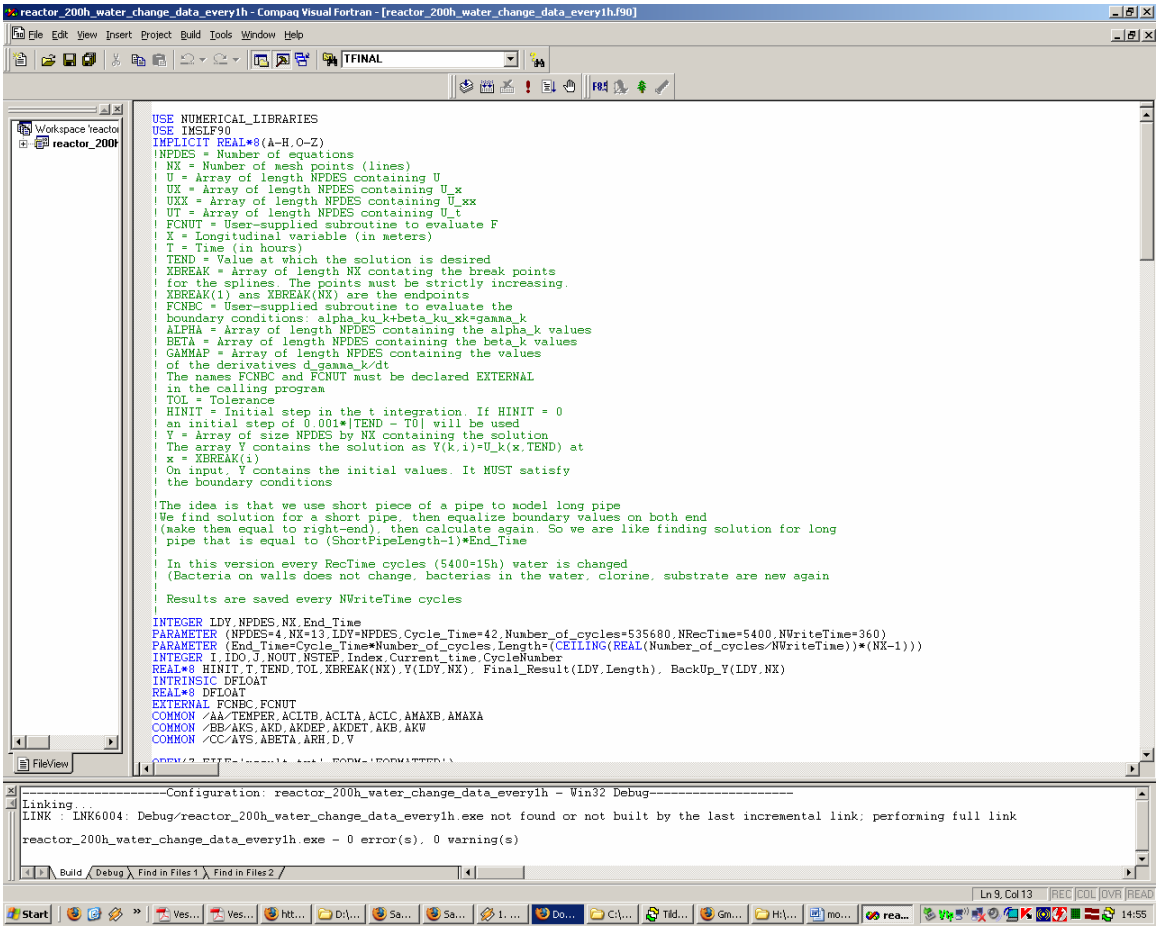

Figure 18. Biofilm regrowth model in Compaq Visual FORTRAN v6.5

The input values for bacterial regrowth simulation were temperature of water, duration time of simulation, constant velocity, constant diameter of pipe, bacteria in bulk water (cells/mL), bacteria in biofilm (cells/cm²), biodegradable organic carbon (BDOC, mg/L), and residual chlorine concentration (mg/L).
The description of the model and processes taken into account in RIGA model is given in Zhang *et al.,* (2004) (Figure 19). The phosphorus recycling is not yet included in the mathematical model.

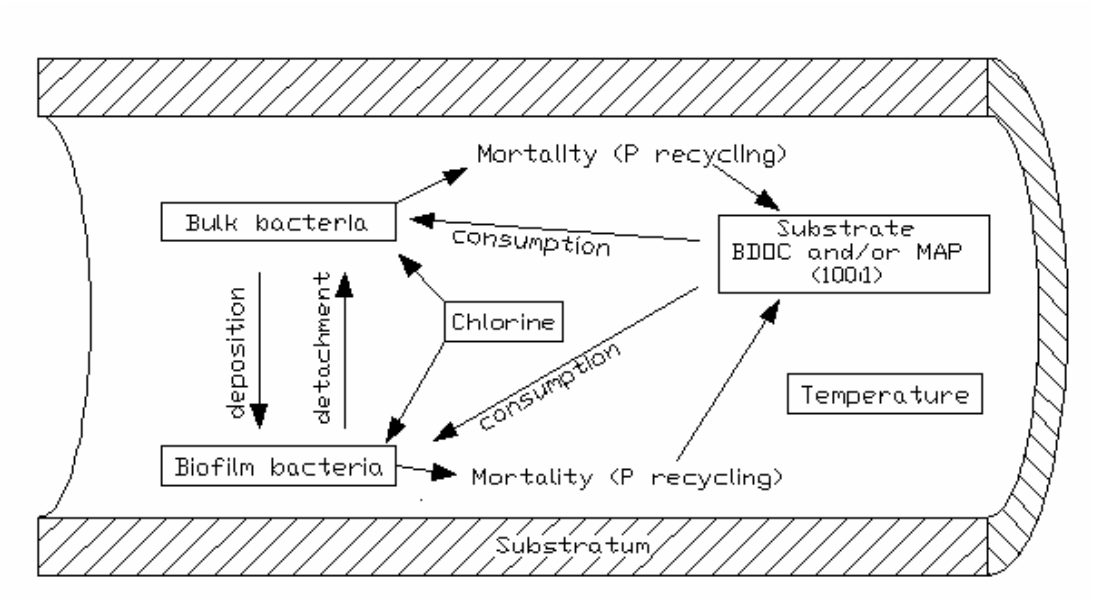

Figure 19. Processes taken into account in biofilm regrowth model

To describe growth dynamic of bulk and biofilm bacteria MONOD kinetic is used (1942). The system of four equations where water temperature, concentration of chlorine and BDOC are assumed to control growth rate, is as follows:

$$\mu_b = \begin{cases} \mu_{\max,b}\left(\frac{S}{S+k_S}\right)\exp\left[-\frac{Cl_2 - Cl_{2,tb}}{Cl_{2,c}}\right]\exp\left[-\left(\frac{T_{opt}-T}{T_{opt}-T_i}\right)^2\right], if\_Cl_2 > Cl_{2,tb} \\ \mu_{\max,b}\left(\frac{S}{S+k_S}\right)\exp\left[-\left(\frac{T_{opt}-T}{T_{opt}-T_i}\right)^2\right], if\_Cl_2 \le Cl_{2,tb} \end{cases}$$

$$\mu_a = \begin{cases} \mu_{\max,a}\left(\frac{S}{S+k_S}\right)\exp\left[-\frac{Cl_2 - Cl_{2,ta}}{Cl_{2,c}}\right]\exp\left[-\left(\frac{T_{opt}-T}{T_{opt}-T_i}\right)^2\right], if\_Cl_2 > Cl_{2,ta} \\ \mu_{\max,a}\left(\frac{S}{S+k_S}\right)\exp\left[-\left(\frac{T_{opt}-T}{T_{opt}-T_i}\right)^2\right], if\_Cl_2 \le Cl_{2,ta} \end{cases}$$

where $\mu_{max,b}$ – maximum growth rate of bacteria in the bulk water; $\mu_{max,a}$ – maximum growth rate of bacteria in biofilm; S – substrate concentration

measured as BDOC; k_s – half-saturation constant of substrate uptake; T_{opt} – optimal temperature for bacterial activity; T_i – temperature dependent shape parameter, which is assumed to be a significant in bacteria growth; T – in situ temperature of water; $Cl_{2,t}$ – threshold above which chlorine affects bacterial activity ($Cl_{2,ta}$ will be greater than $Cl_{2,tb}$ because biofilms are more resistant to inactivation by chlorine); $Cl_{2,c}$ – characteristic chlorine concentration defining the rate of decreases of bacterial activity with increasing chlorine concentration (when $Cl_2 > Cl_{2,t}$, μ_b decrease exponentially due to chlorine concentration, but when $Cl_2 \leq Cl_{2,t}$, μ_b was not depend on concentration of chlorine); Cl_2 – in situ residual chlorine concentration.

The material balance for free bacteria is described by the equation:

$$\frac{\partial X_b}{\partial t} = -v\frac{\partial X_b}{\partial X} + D\frac{\partial^2 X_b}{\partial X^2} + \mu_b X_b + K_{\det} X_a \frac{v}{R_h} - k_d X_b - k_{dep} X_b \qquad (1)$$

Unlike free bacteria, the material balance to describe attached bacteria does not depend on transport of water in the bulk flow. The descriptions of growth rate and mortality rate of attached bacteria are similar to those of free bacteria, except $Cl_{2,t}$ (see above). The material balance for biofilm bacteria is described as follows:

$$\frac{\partial X_a}{\partial t} = \mu_a X_a - k_{\det} v X_a - k_d X_a + k_{dep} R_h X_b \qquad (2)$$

The material balance to account for loss of substrate from bulk water includes substrate utilization by both the free and attached bacteria expressed as:

$$\frac{\partial S}{\partial t} = -v\frac{\partial S}{\partial X} + D\frac{\partial^2 S}{\partial X^2} - \frac{1}{Y_R \beta}\left(\mu_a \frac{X_a}{R_h} + \mu_b X_b\right), \qquad (3)$$

where Y_g – growth yield coefficient of bacteria; β – number of bacteria that are produced for each milligram of organic carbon in cell biomass.

Chlorine disappears from bulk solution both by oxidation reactions in the bulk water which are assumed to follow first-order kinetics with respect to Cl_2 and at the surface of pipe walls which are assumed to be zero order, i.e., independent of Cl_2 concentration in the bulk water. The resulting material balance for chlorine is:

$$\frac{\partial Cl_2}{\partial t} = -v\frac{\partial Cl_2}{\partial X} + D\frac{\partial^2 Cl_2}{\partial X^2} - k_b Cl_2 - \frac{k_w}{R_h}, \qquad (4)$$

where k_b – first-order kinetic constant for chlorine decay in bulk water and k_w – zero-order rate constant for the wall reaction.

Values of all parameters used in the bacterial regrowth model simulations are listed in Table 3.

Table 3. Values of Parameters in Regrowth Model

Parameter/Input variable	Symbol	Value	Unit	Reference
Maximum regrowth rate of free bacteria	$\mu_{max,b}$	0,20	h^{-1}	Camper 1996
Maximum regrowth rate of attached bacteria	$\mu_{max,a}$	0,20	h^{-1}	Camper 1996
Optimal temperature for bacterial activity	T_{opt}	25	^oC	Dukan *et al*. 1996
Temperature dependent shape parameter	T_i	15	^oC	Dukan *et al*. 1996
Chlorine threshold for free bacteria	$Cl_{2,t,b}$	0,03	mg/L	Laurent et. al. 1997
Chlorine threshold for attached bacteria	$Cl_{2,t,a}$	0,10	mg/L	Laurent et. al. 1997
Characteristic chlorine concentration	$Cl_{2,c}$	0,20	mg/L	Laurent et. al. 1997
Monod half saturation coefficient	K_s	0,4	mgC/L	Laurent et. al. 1997
First-order kinetic constant for detachment	k_{det}	0,03	h^{-1}	Bois et. al. 1997
First-order kinetic constant for deposition	k_{dep}	0,25	$h^{-1}(m/s)^{-1}$	Bois et. al. 1997
Bacterial mortality rate	k_d	0,06	h^{-1}	Laurent et. al. 1997
Growth yield coefficient for bacteria	Y_g	0,15	mg/mg	Laurent et. al. 1997
First-order kinetic constant for chlorine decay by bulk demand	k_b	0,03	h^{-1}	Zhang and DiGiano 2002a
Zero-order kinetic constant for chlorine decay by wall demand	k_w	26	$mg/m^2/h$	Zhang and DiGiano 2002a
Constant of bacteria that are produced for each mg of carbon in cell biomass	β	10^{10}	cell/mgC	Hammes and Egli, 2005

3.2 Computational model for PropellaTM

Before all model constants are verified the modelling process should be concentrated for biofilm reactor PropellaTM. The system of equations (1) – (4) subject to initial and boundary conditions is solved numerically by means of IMSL routine MOLCH which solves a system of partial differential equations by the method of lines. The solution is represented by cubic Hermite polynomials.

The model developed by Zhang *et al*. (2004) is valid for straight long pipes (or straight long pipe segments) where the size of the pipe in the radial direction is much smaller than the size of the pipe in the longitudinal direction. As a result, Zhang *et al*. (2004) neglected the dependence of the solution on the radial coordinate. There are two major differences between Zhang's model

(2004) and the model used for the PropellaTM. First, the characteristic size of the PropellaTM reactor in the radial direction is comparable to that in the longitudinal direction. Second, water is circulating in the PropellaTM reactor while water is passing only once through the pipe (or system of pipes) in Zhang's model. The modelling approach used for PropellaTM is briefly described below.

It is clear that the inclusion of terms depending on the radial coordinate would certainly improve Zhang's model. However, keeping in mind that (1) there are many constants in the Zhang's model such that their values are known only approximately and (2) the addition of the radial coordinate would increase the number of constants whose values may be not known at all, we decided to neglect the dependence of the flow characteristics on the radial coordinate. Thus, a balance is sought between the complexity of a model and its ability to reproduce experimental data with minimum required empirical information. Of course, the validity of this assumption can only be tested when more advanced models would be available.

In order to take into account water re-circulation in the PropellaTM reactor, boundary and initial conditions are modified as follows. Suppose that the length of the PropellaTM reactor is L so that the longitudinal coordinate varies from $x = 0$ to $x = L$. During the first run the boundary conditions at $x = 0$ are fixed, all the functions in (1) – (4) are specified at certain levels (in fact, the boundary conditions from Zhang's paper are used). The derivative of all functions at $x = L$ is assumed to be zero. The initial conditions are also specified as in Zhang's paper. After the first run the solution at all grid points is known. This solution is considered as initial condition for the next run. In addition, the conditions at $x = 0$ for the next run are assumed to be equal to the conditions at $x = L$ from the previous run. The procedure is repeated many times (this is how re-circulation is modelled for PropellaTM).

The computation time of 30 day simulation for such a model takes time up to 2 h using an 2,01 GHz AMDAthlonTM 2.0 GB of RAM PC computer.

3.3 Comparison of both models for straight pipe and for biofilm reactor PropellaTM

For modelling of biofilm regrowth two types of approaches are used: model of perfectly mixed with water recycling (PMWR) Propella TM reactor and model for long straight pipe. Although flow conditions in both models are different both have been used to investigate biofilm formation in the real distribution networks. There are at least two major differences between PMWR and pipe model. Firstly, boundary conditions used for a pipe are not applicable for PMWR, as water in reactor is recycled while water in a pipe is constantly replaced by a fresh one. Secondly, due to a limited length of PMWR reactor, the assumption of fully developed flow used in Zhang *et al*. (2004) may be violated. If the flow is fully developed then the velocity vector has only one nonzero longitudinal component which is independent on a radial coordinate. Unlike in pipe, the flow in PMWR models is not one-dimensional.

For simulation of PropellaTM reactor the solution is obtained for 0.1 m/s flow of water in a 1 m long pipe (one cycle in a 500-mm PropellaTM reactor). The selected simulation period is equal to 10 min., this is the time needed for water to make a completed loop. The initial and boundary conditions are:

$$\begin{array}{lll} X_a(0,t)=0 \ \text{cell/cm}^2 & \dfrac{\partial X_a(1,t)}{\partial x}=0 & X_a(x,0)=0 \\ X_b(0,t)=10 \ \text{cell/mL} & \dfrac{\partial X_b(1,t)}{\partial x}=0 & X_b(x,0)=0 \\ Cl_2(0,t)=1 \ \text{mg/L} & \dfrac{\partial Cl_2(1,t)}{\partial x}=0 & Cl_2(x,0)=0 \\ S(0,t)=0.4 \ \text{mg/L} & \dfrac{\partial S(1,t)}{\partial x}=0 & S(x,0)=0 \end{array} \quad (5)$$

After the simulation of a one single loop was completed, the boundary values on the right side were copied to the left side, thus, representing a closed cycle. Then, a simulation for another 10 min. was performed and data for next cycle were acquired. This process was repeated 7200 times, so 200 hours of water flow in PropellaTM reactor were simulated. For comparison, data of the last 100 cycles (last 100 meters of flow) were taken. For pipe simulation the solution was obtained for the same flow velocity and pipe diameter for 100 m long pipe. The initial and boundary conditions were the same as in (5).

As it has already been stated above, there are differences between flow models in the PropellaTM reactor and in the pipe. Water in the pipe is continuously replaced and the boundary conditions remain unchanged during the whole simulation period. In the PropellaTM reactor water is flowing in a closed loop, and is replaced after every 15 h during the whole simulation period, and boundary conditions change at each simulation cycle.

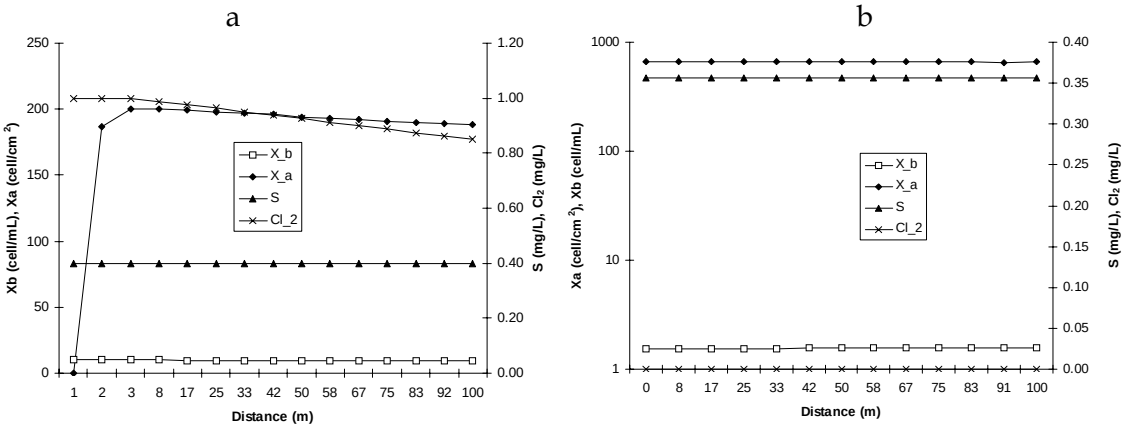

Figure 20. Results from bacteria regrowth simulation in (a) pipe and (b) in PropellaTM (as PMWR type of reactor) (Juhna *et al.*, 2007).

Notes: (Y-axis in log scale, on X-axis the last 100 m are shown); X_b- bacterial number in water, X_a- bacterial number in biofilm, S- substrate (biodegradable organic carbon), Cl_2 – residual chlorine concentration.

Juhna *et al.*, (2007) in Figure 20 presents results of 200 h bacteria growth simulation in the 100 meter long pipe and in the PropellaTM reactor (as PMWR reactor). Biofilm bacteria concentration (X_a) goes to zero at the left side of the pipe (Figure, 20a) according to the boundary value. The constant bacterial number is reached after about 3m in pipe reactor. Further all values are relatively constant except concentration of chlorine. Mathematical models showed that the chorine concentration decreases gradually from 1 mg/L to about 0.85 after 100 m of water passage in the pipe, whereas in PropellaTM, the chlorine level dropped below 0.1 mg/L after 2.5 hours during every cycle (data not shown). This can not be observed in Figure (20b) as only the last 100 m of water passage through the reactor are shown. It is because water in the PropellaTM reactor is replaced every 15 hours whereas simulation results are shown for the last minutes of experiment when chlorine is completely consumed. These results agree well with data observed in the full scale studies (Zhang and DiGiano, 2002b). The model also shows that bacteria number in biofilm (X_a) in reactor with high concentration of chlorine is lower: in PropellaTM bacteria number in biofilm was about 5 times higher than in the pipe. This could be explained by the inhibitory effect of chlorine on bacterial growth. In turn, the higher total (biofilm and water phase) bacterial number in PropellaTM leads to lower concentration of substrate, because; in the reactor with higher bacterial number, more of substrate is consumed by bacteria.

In general, the results from model simulation are in agreement with a common understanding of behaviour of bacteria in drinking water distribution networks. The tendency of bacterial growth was similar in both types of models; however the absolute values were different.

3.4 Results of simulation for PropellaTM reactor of RIGA model

The simulations were produced for model of biofilm reactor PropellaTM. Simulations conditions: simulation time 1440 hours (60 days); water flow velocity: 0.25 m/s; bulk water bacteria concentration in influent of reactor: X_b=1000 cells/mL; biofilm bacteria concentration on surface of reactor surface: X_a=0 cells/cm^2; concentration of BDOC (S), *in situ* temperature (T) and concentration of residual chlorine (Cl_2) was changed according necessity (see above).

3.4.1 Checking of effect from maximum growth rate of biofilm bacteria

There was changed coefficient of maximum growth rate of attached bacteria $\mu_{max,a}$. The original value 0.2 h^{-1} was changed to 2 and 20 h^{-1} and reasonable increases of concentration of biofilm and bulk water bacteria were not detected. These tendencies can be supposed to be logical.

3.4.2 *Checking of in situ temperature, substrate and chlorine concentration effect*

In a Table 4 are given five different scenarios of simulation for bacterial growth model of PropellaTM reactor.

Table 4. Input values of biofilm regrowth model parameters for some simulation scenarios for PropellaTM reactor

	1.	2.	3.	4.	5.
Xb, cells/mL	1000	1000	1000	1000	1000
Xa, cells/cm^2	0	0	0	0	0
T, ^{o}C	5	5	25	15	25
BDOC, mg/L	0.2	0.2	5	2	5
Cl_2, mg/L	0	1	0	0.5	1

Simulation results showed that increase of temperature and substrate concentration significantly increases biofilm and bulk water bacteria concentration. Concentration of biofilm bacteria was for 3-logs and for bulk water bacteria for 2-logs higher in 3.simulation than in 1.simulation.

The concentration of biofilm bacteria for 1. and 3.simulations was same, but for 3. and 5.simulations – almost same. The concentration of biofilm bacteria for 4.simulation was higher than for 1. and 2.simulations and lower than for 3. and 5.simulations which is logical result.

Increases of chlorine concentration didn't affect biofilm bacteria concentration (1. and 2.simulations) in case when temperature and substrate concentration were low. But for situations when *in situ* temperature and BDOC concentration were high (3. and 5.simulations) – increase of chlorine residual from 0 to 1 mg/L affects biofilm bacteria concentration only in beginning of first simulation cycle, further decreases of biofilm bacteria concentration was not detected. This phenomena cannot be explained while in operation conditions of PropellaTM reactor in this model was assumed, that amount of water was changed (and chlorine concentration restored) after every 15 hours. From engineering point of view one of the reasons can be resistance of biofilm bacteria, which increases with development of biofilm layer.

3.4.3 *Checking of substrate and chlorine concentration effect at 25º and 6ºC*

In a Table 5 are given scenarios of simulation for bacterial growth at high and low water temperature.

Table 5. Input values of biofilm regrowth model parameters for some simulation scenarios for PropellaTM reactor at high (25^o) and low (6^{o}C) temperature

	Norm.	BDOC	Cl
Xb, cells/mL	200000	200000	200000
Xa, cells/cm^2	0	0	0
BDOC, mg/L	0.4	1	0.4

Cl_2, mg/L	0.3	0.3	1

There is no reasonable effect of increase of nutrients and chlorine concentration on biofilm and bulk water bacteria concentration at T=6°C.

Increase of chlorine concentration lead to negligible decrease of biofilm bacteria at T=25°C, and increase of substrate gives increase of biofilm bacteria within 1-log at T=25°C.
Comparison of simulation results for conditions „Norm." at T=25°C gives more bacteria than at T=6°C and increase of bacteria concentration is more reasonable for biofilm bacteria than for bulk water. *In* situ temperature T=25°C for both biofilm and bulk water bacteria concentrations gives increases within 1-log.

3.5 Results of simulation for straight pipe of RIGA model

The simulations were produced for 5 km long pipe; simulation time: 720 hours (30 days); water flow velocity: 1 m/s; water temperature: T=20°C; bulk water bacteria concentration in effluent from treatment plant: Xb=1000 cells/mL; biofilm bacteria concentration on DS pipe surface: Xa=0 cells/cm²; concentration of BDOC in effluent from treatment plant: S=1 mg/L; residual chlorine concentration in effluent from treatment plant: Cl_2=0.2 mg/L (some of these parameters during simulation were changed, that thus should be mentioned further).

3.5.1 Checking of velocity significance

There was observed increase of concentration of bacteria in biofilm and in bulk water with increase of flow velocity. The similar positive relationship was observed between flow velocity and concentration of biofilm bacteria when BDOC concentrations was set for S=1 mg/L and 0.2 mg/L. As well logical tendencies of flow velocity and concentration of biofilm bacteria were observed at T=20°C and 10°C.
There was observe paradox that with substrate concentration S=0.5 mg/L, when biofilm concentration was higher at flow velocity 0.5 m/s than at flows 0.2 un 1 m/s.

There was observed interruption of simulation (error) when at water flow velocity 0.1 m/s, concentration of biofilm bacteria in the start of simulation was set greater than, 100 cells/cm². The same interruption of simulation (error) was observed at water flow rate 0.2 m/s and biofilm greater than 1'000'000 cells/cm².

3.5.2 Checking of biofilm bacteria effect

There was compared two situation when the bacteria concentration of biofilm at start of simulation is 0 cells/cm² and simulation when concentration is greater than 0 cells/cm². For both cases there was observed the same concentration biofilm and bulk water bacteria with one exception for first 170

m of pipe, when simulation with any concentration of biofilm bacteria at start lead to sharp decreases of biofilm bacteria concentration.
This indicated that there is no influence on model results with addition of concentration of biofilm bacteria on the start of simulation.

3.5.3 Checking of dispersion coefficient significance

The results of simulations showed that increases of dispersion coefficient from 18 to 180 and 1800 m^2/h didn't affect levels of Xb, Xa, S, and Cl_2.

3.5.4 Checking of effect from maximum growth rate of biofilm bacteria

The results of simulations showed that increases of maximum growth rate of attached bacteria $\mu_{max,a}$ from the original value 0.2 h^{-1} to 2 h^{-1} and 20 h^{-1} gives negative values ("- …… cells/cm^2 ") of biofilm bacteria concentration. The simulation results
The increase of growth rate from 0.2 h^{-1} to 0.4 h^{-1} gives logical tendencies. The concentration of biofilm bacteria increase for 1 to 2-logs. The negative values can be observed with growth rate 0.5 h^{-1} and higher.

3.5.5 Checking of effect for number of cycles

The increase of number of cycles from 24 to 120 and 600 didn't change the results of simulation.

3.5.6 Checking of substrate concentration effect

The changes of BDOC concentration lead to logical tendencies. More substrate gives more bacteria concentration in both biofilm and bulk water. The increases of BDOC from 0.1 mgC/L to 1 mgC/L give increases of biofilm bacteria more than for 3-logs. The further increase of BDOC to 1.8 mgC/L gives increases of biofilm bacteria for additional 1-log. The BDOC concentration 2 mgC/L and more give negative values of biofilm bacteria concentration.

3.5.7 Checking of in situ temperature effect

The increase of temperature gives increases of biofilm bacteria concentration with maximal values at T=25°C, the further increases of temperature gives decreases of biofilm bacteria concentration. At T=10°C and T= 15°C the concentration of biofilm bacteria decreases and BDOC almost was not consumed. At T=20°C was observed significant increases of concentration of biofilm bacteria and consumption of BDOC.
There was observed the same concentrations of biofilm bacteria for conditions when temperature differs from T=25°C by the same gap on both directions. This means that there was similar biofilm bacteria concentrations for both T=20°C and 30°C as well for T=10°C and 40°C.

3.5.8 Checking of chlorine concentration effect

The changes of chlorine concentration lead to logical tendencies. More chlorine gives fewer bacteria. The chlorine concentration at the start of simulation less than 0.1 mg/L gives negative values of biofilm bacteria concentrations.

3.5.9 Checking of bulk water bacteria concentration effect

The increases of bacteria concentration in bulk water (10; 1000; 100'000; 1'000' 000 cells/mL) lead to increases of both biofilm and bulk water concentrations. Theses increases in absolute values are negligible. During these simulations the BDOC concentration tends to level of 0.2 mgC/L, but there was not observed total consumption of BDOC.
There was an exception that at bulk water concentration 1'000'000 cells/mL there was significant increases of bacteria concentration in both biofilm and bulk water.
The bulk water concentration 1 cell/mL gives negative values of biofilm bacteria, which disappears when bulk water concentration of the start of simulation was set 6 cells/mL, this concentration should be taken as minimal necessary concentration to get useful results.

There was observe paradox that biofilm bacteria concentration after simulation in stationary phase was little bit higher for entered input values as 10 cells/mL than for 1000 cells/mL at the start of simulation.

3.6 Conclusions

In overall RIGA model showed tendencies which are logical and in accordance with literature mentioned data.

In accordance of several research projects by TZW it was shown that the **main part of the bacteria in the DSs was fixed within biofilms**, which is congruent with the results by other authors (Servais *et al.*, 1992, Flemming *et al.*, 2002). Therefore, the portion of bacteria in the bulk water was relatively low under usual conditions. Using a supply pipe of 100 mm in diameter, a usual bacteria concentration in the bulk water of 50' 000 cells/mL and a bacteria concentration of 1'000' 000 cells/cm² at the pipe surface, there were 87.5 % of the bacteria in the biofilm and 12.5 % in the bulk water. Moreover, the portion of colony-forming units in the biofilm was 98.8 % when HPC determined by the GDWR of 5 cfu/mL in the bulk water and a density of 1000 cfu/cm² in the biofilm were compared.

RIGA model is in agreement with this statement and although model accounts for TBN (not HPC) the proportion of biofilm bacteria is in range 95-99% for all bacteria presented in distribution pipeline.

According to findings of Wolf (2002) **the growth of HPC (GDWR) was found almost exclusively on surfaces**. He conducted extensive studies at TZW Dresden in order to explain the growth of colony forming units. As shown in

Figure 21, there was a significant correlation between the bottle size and HPC in regrowth experiments (7 days of sample incubation at 15°C, afterwards determination of HPC (GDWR 20°C)) in different drinking water samples. The surface-area to volume ratio decreases with increasing bottle size, therefore the growth of colony forming units happens mainly on the surfaces, whereby high HPC in the bulk water result from a release of bacteria into the water during growth on the surface.

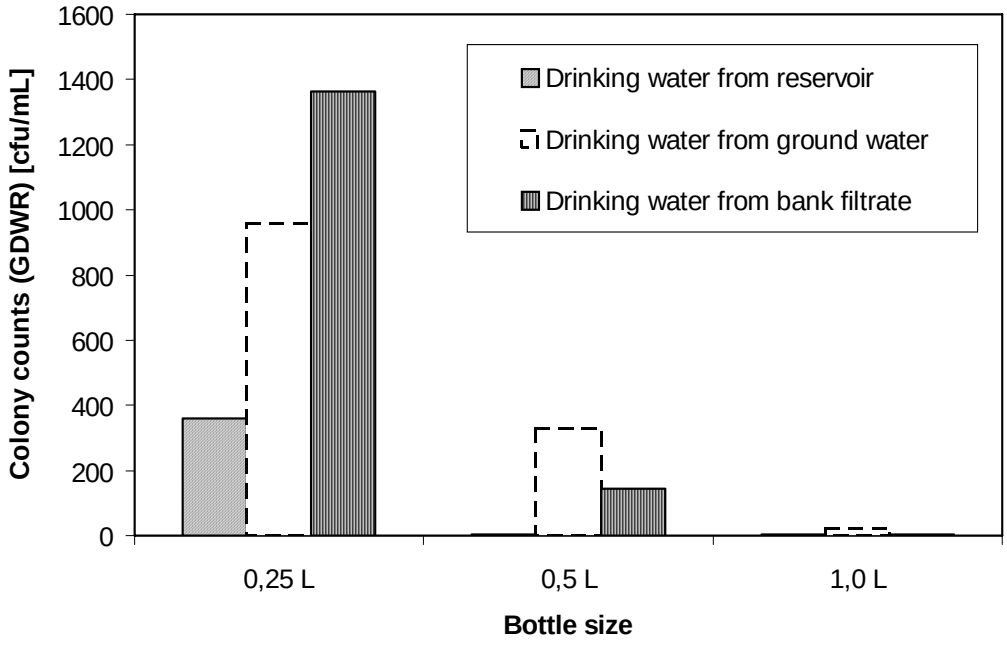

Figure 21. Influence of bottle size on HPC in laboratory regrowth experiments

How stated earlier the RIGA model gives major part of bacteria on surface of pipes that is defined with two approaches:

i) equations describing growth dynamic of bacteria by Monod kinetic foreseen that biofilm bacteria is more resistant to chlorine inhibitory effect than bulk water bacteria (see coefficients $Cl_{2,t,b} < Cl_{2,t,a}$);

ii) in the the material balance equations (1) and (2) is assumed that bacteria deposition rate is higher than detachment rate.

Further experiments are necessary to determine the coefficients of yield which complement findings by TZW which indicate that bacteria growth happens exclusively on surface. At the moment coefficients which predicts bacteria yield (Yg and β on equation Nr.3) are equal for both biofilm and bulk water bacteria.

As it was stated in recent findings by Wricke *et al*. (2002b), Korth *et al*. (2004), Korth and Wricke (2001 and 2004b) a stable bacteriological situation will be found under stable preconditions in the network. This statement is confirmed with studies in distribution networks. Figures 22 show the results of perennial studies in a long-distance DS. Neither a significant change of HPC (GDWR 20°C) nor a change of TBN was detected in the examined part of the pipe (length approx. 80 km). In this case, disinfection residuals were

relatively low in the finished water (0.2 mg/L chlorine + 0.2 mg/L chlorine dioxide). Afterwards, there was no disinfection carried out within the network. Fluctuations in the water quality were relatively low in this water works.

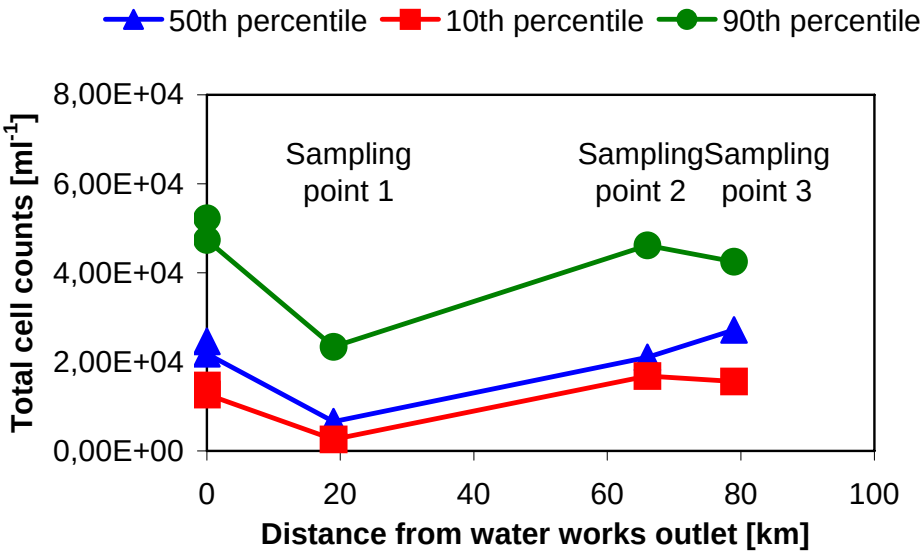

Figure 22. Change of TBN in a long-distance DS (Wricke *et al.*, 2002b).
Note: cells stained with DAPI day.

The German researchers found that **elevated HPC (GDWR) were found if the equilibrium of biofilm influencing processes was disturbed or hindered**. Therefore, more cells are released from the biofilm into the water. The main reasons for this are changes in nutrient concentration, fluctuating disinfection residuals and, assumedly, temperature changes.

These statements should be included in RIGA model in future, while at the moment model predicts biofilm regrowth in stable conditions. As well material balance equation (Nr.2) at the moment is not dependant from water velocity. Therefore, the model must be improved with dynamic conditions when variations of water flow and water quality changes entering the DS in time.

It can be concluded that the microbiological water quality in the distribution network is affected by processes in the biofilm (detachment from the biofilm, attachment of bulk water cells on surfaces and bacteria mortality in the water). The equilibrium of the processes is reached due to stable conditions resulting in stable bacteriological parameters in the network. However, changes in the microbiological water quality as seen in an increased bacteria release from the biofilm can be found as a result of disturbances of the biofilm caused by changes in the water quality. The main factors for such disturbances are fluctuations in nutrient concentrations and disinfection residuals as well as probable water temperature fluctuations. The structure of the biofilm determines the level of the impact on the water quality.

Nevertheless, the typical parameters of a biofilm responsible for its sensitivity to disturbances remain as yet unknown. Therefore, the existing conceptual models only describe the correlation between the changes in the water quality parameters and the bacteriological quality changes. In order to concretize this conceptual model it would be necessary to explain the connection between biofilms and their sensitivity to disturbances which result in an elevated bacteria release. Further examinations are necessary for verifying these findings in order to be included into the mathematical model which will be developed within this project.

3.7 RIGA model improvements

- The biofilm regrowth model in a future should contain improvements: the coefficients of yield, which in this mathematical model are same as for suspended and attached bacteria (see equation Nr. 3 and Table 3). Higher yield for suspended bacteria (more bacteria produced from the same amount of carbon) than for biofilm bacteria will be assumed in the model. As well additional coefficients of yield for new equation system of phosphorus balance (see further suggestion).
- The maximum growth rate different for suspended and attached bacteria (equation system of growth rate and equation 1, 2, and 3).
- Model should contain two possible scenarios for bacterial regrowth. According to the nutrients ratio in bulk water growth rate-limiting substrate could be either carbon or phosphorus. Optimal ratio of carbon, nitrogen and phosphorus is about 100:10:1. Therefore equation system of growth rates should contain option of phosphorus influence and there should be additional equation for phosphorus utilization like equation Nr.3.
- New mass balance equation for phosphorus utilization should contain option for phosphorus recirculation. Dissolved phosphorus regeneration in range of 210-885 pM h^{-1} is well established in limonological environments (Hudson *et al.*, 2000), thus models allow to test this phenomena in water supply systems. And additional coefficient of phosphorus release from pipes should be tested.
- Wall reaction of chlorine due to pipe material (equation Nr. 4). Cast iron pipe corrosion should be updated.
- Threshold values for chlorine influence on suspended and attached bacterial activity $Cl_{2,t}$ (equation system of growth rate).
- Suspended particle accumulation and transport in the DSs should be included (equation Nr.1.).
- Bacteria release from pipe surface should be linked with water quality, concentration of chlorine and temperature disturbances.
- The model should be complement with dynamic conditions in time.

3.8 References

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4 CONCLUSIONS

As it is in Techneua project Deliverable D5.6.1 and D5.6.2 "Evaluation report on operational methods and maintenance schemes – Applied in praxis and compared to best practice" within the water safety planning processes it is important for water companies to develop a more forward looking approach to managing water quality by predicting when and where events are likely to occur rather than responding to events after they occurred. Therefore within this WP we have produced a bacterial regrowth model which should be used as a tool for predicting of water quality deterioration.

The statistical models (data driven) like proposed by Zhang and DiGiano (2002) are not useful for this reason because they often are produced from one specific site and equation found from regression analysis gives result useful only for current conditions. Therefore literature was analysed to found deterministic models which can predict bacterial regrowth. Some of them are very well developed and verified in DSs: SANCHO model (Servais *et al* 1995), PICCOBIO model (Dukan *et al* 1996, Piriou *et al*. 1998). Most of the models are proprietary object therefore they are not useful for further improvements. As well they have several drawbacks – biological processes are not linked with hydraulic model, there are to much specific constants which made a model weaker.
A model proposed by Zhang *et al*. (2004) was taken as a base to develop useful tool for bacteriological water quality prediction. In this model offered approaches in form of equations were written in FORTRAN programme and tested for reproducibility, which showed good response for several possible scenarios, like effect of increases of substrate (BDOC), disinfectant (chlorine residual), temperature and other parameters and constants included within model.
Model was written for two different approaches: one as for long straight pipe, another one for biofilm reactor PropellaTM. Reactor PropellaTM is useful tool for experimental action to growth biofilm and to verify in future model constants. There was made simulations for comparability of results for both types of models and data in general showed similar trends of development of biofilm.
However there is necessity to made improvements of both models to attain more stable simulation results, because some times model simulations is interruption, which can be explained by using of no applicable input values for existing model.
In the future the model for straight long pipe should be expanded to several pipe systems. As well model constants should be tested within reactor PropellaTM and after that approved and used in model. Some of these experiments according to project action plane should be produced within 21 months of project.
The verifiction of the proposed conceptual model of bacterial regrowth should be linked with network model application. The network model includes a hydraulic model and an event-driven (Lagrangean) advection and mixing model, for an unlimited array of simultaneous parameter

concentrations, as well travel time and source trace. This solution is based on the Epanet 2.0 toolkit for the hydraulic solution, and superimposes its own water quality engine.

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