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Factors affecting migration of bioavailable compounds investigated by batch set-ups and continuous flow model systems

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Publication date:
2004

Document Version
Publisher's PDF, also known as Version of record

Environment & Resources
Technical University of Denmark

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Charlotte Bettina Corfitzen
Ph.D. Thesis, June 2004
Environment & Resources DTU
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Cover: Birte Brejl
Printed by: DTU tryk
Environmental & Resources DTU
ISBN 87-89220-86-2

The thesis will be available as a downloadable pdf-file from the department's homepage on: www.er.dtu.dk

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PREFACE

The Ph.D. work presented here has been performed at Environment & Resources DTU at the Technical University of Denmark. The project was granted as a two year project period, which was performed within the period from November 2001 to June 2004, with the inclusion of previous work representing a one-year workload. The Ph.D. was partly financed by the Danish EPA and by a project under the European Acceptance Scheme: Assessment of the microbial growth support potential of construction product in contact with drinking water (CPDW) – Development of a harmonised test to be used in the European Acceptance Scheme concerning CPDW – EVK1-CT2000-00052.

The thesis consists of a summary of project related literature and four papers: two manuscripts and two articles prepared for submission to international peer-reviewed periodicals. The papers are not included in the this electronic version, but can be obtained from the Library at Environment & Resources DTU, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@er.dtu.dk).

I) Corfitzen, C.B.; Albrechtsen, H.-J. (2004a)
Development of a continuous flow model system for studies of biofilm formation on polymers and its application on PVC-C and PVC-P
Article prepared for submission

II) Corfitzen, C.B.; Arvin, E.; Albrechtsen, H.-J. (2004)
Processes influencing migration of bioavailable organic compounds from polymers - investigated during biotic and abiotic testing under static and non-static conditions with varying S/V-ratios
Article prepared for submission

III) Corfitzen, C.B.; Albrechtsen, H.-J (2004b)
Investigating aftergrowth potential of polymers in drinking water – the effect of water replacement and temperature
Manuscript

IV) Corfitzen, C.B.; Albrechtsen, H.-J. (2004c)
Optimisation of ATP determination in drinking water
Manuscript

The PhD work has included participation in the preparation of three project reports, a conference proceeding and a conference poster, which are not included here:

van der Kooij, D.; Albrechtsen, H.-J.; Corfitzen, C.B.; Ashworth, J.; Parry, I.: Enkiri, F.; Hambsch, B.; Hametner, C. Kloiber, R.; Veenendaal, H.R.; Verhamme, D.; Hoekstra, E.J. (2003)
Assessment of the microbial growth support potential of construction product in contact with drinking water (CPDW) – Development of a harmonised test to be used in the European Acceptance Scheme concerning CPDW – EVK1-CT2000-00052, 103 pages and appendixes

Corfitzen, C.B.; Albrechtsen, H.-J.; Arvin, E.; Jørgensen, C. (2002)
Release of organic compounds from polymeric materials – microbial growth
In Danish, Report to the Danish EPA, 87 pages and appendixes
(Miljøprojekt nr. 718, Miljøstyrelsen)

Jørgensen, C.; Albrechtsen, H.-J.; Arvin, E; Corfitzen C.B. (2002)
Investigation of bacteria numbers and aftergrowth potential in treated water
In Danish, Report to the Danish EPA, 59 pages and appendixes
(Miljøprojekt nr. 719, Miljøstyrelsen)

Albrechtsen, H.-J.; Corfitzen, C.B.; Arvin, E.; Jørgensen, C. (2000)
Microbial growth on organic compounds released from polymers in distribution systems
In Danish, In Proceedings of the 2nd Nordic Water Supply Conference, June 2000, Helsingør, Denmark

Poster:
Corfitzen, C.B.; Albrechtsen, H.-J.; Arvin, E.; Jørgensen, C. (2001)
Factors affecting quantification of assimilable organic carbon (AOC) release from polymers in drinking water systems
IWA 2nd World Water Congress, 15-19 October 2001, Berlin, Germany

The efforts of my supervisors Associated Professor Hans-Jørgen Albrechtsen and Professor Erik Arvin at Environment & Resources to get this project initialised are greatly appreciated. Their commitment and interest have positively influenced the full course of the project.

A number of people at Environment & Resources are recognised for their assistance with the practical work and their contribution to creating an enjoyable work environment, especially Mona Refstrup, Denny Viholt, Uwe Wolter, Margrethe Sørensen, Bithe Ebert, Bent Skov and Henriette Blankenstjerne. Also Torben Dolin and Birthe Brejl, who made the figures for the papers and this thesis.

Also thanks to Richard Horne Hansen and the rest of the staff at Lyngby water works for showing interest and providing ongoing access to freshly produced water, which has played an important part as test water in the practical work.

I owe a lot of gratitude to my loving family (on both the Danish and German side of the boarder) and friends for their continuously support and acceptance of my absence and lack of involvement during the last part of the project period.

Especially thanks to my wonderful fiancé Gerald Heinicke for proofreading, constructive criticism and suggestions during the writing of the thesis. Thank you for all your support, love and encouragement – especially during the last months.

Charlotte Bettina Corfitzen
June 2004

ABBREVIATIONS

Al/PEMD:	PEMD internally reinforced with aluminium foil
AO:	Acridine Orange
AOC:	Assimilable Organic Carbon
ATP:	Adenosine TriPhosphate
BFP:	Biofilm Formation Potential
BFR:	Biofilm Formation Rate
BPM:	Biofilm Potential Monitors
BPP:	Biomass Production Potential
C:	Carbon
CEN:	European Committee for Standardization
CFU:	Colony Forming Unit
DAPI:	4,6-diamidino-2-phenylindole
DNA:	DeoxyriboNucleic Acid
EPS:	Extracellular Polymeric Substances
GAC:	Granular Activated Carbon
HPC:	Heterotrophic Plate Count
MDOD:	Mean Dissolved Oxygen Demand
MIC:	Microbial Induced Corrosion
N:	Nitrogen
NOX:	*Aquaspirillum* sp. strain NOX
NVOC:	Non-Volatile Organic Carbon
P:	Phosphorus
P17:	*Pseudomonas fluorescence* strain P17
PE:	Polyethylene
PEHD:	High density PE
PEMD:	Medium density PE
PEX:	Cross-linked PE
PP:	Polypropylene
PVC:	Polyvinylchloride
PVC-C:	Chlorinated PVC
PVC-P:	Plasticized PVC
R_2A:	Oligotrophic HPC media developed for drinking water
S/V-ratio:	(Polymer) Surface area to water volume ratio
SP:	Slime Production test
SS:	Stainless Steel
uPVC:	Unplasticized PVC
UV:	Ultraviolet (radiation)

SUMMARY

Polymeric materials are to an ever increasing extent replacing the traditionally used materials in drinking water distribution systems. The polymers are preferred since they are more flexible, less subjected to breakage and constructions cost are lower compared to the traditional materials. However, migration of bioavailable organic compounds from the materials can cause elevated bacterial numbers in the distribution systems. Although bacteria always will be present in distributions systems as planktonic cells and as a biofilm of surfaces, elevated bacterial numbers are undesirable since they can cause operational, hygienic and esthetical problems.

The purpose of the study was to enhance the understanding of how to investigate polymers aftergrowth potential, in order to gain better insight into how polymers affect bacterial levels in distribution systems.

Both an abiotic batch set-up, extracting the materials under sterile conditions and a biotic batch set-up, incubating the materials in the presence of an active biomass, were applied in the investigations. In the abiotic test, the migration of bioavailable compounds was measured as AOC_{P17} in the water phase after removal of the material. In the biotic test, the migration of bioavailable compounds was measured as biomass production in the water phase and on the material surfaces determined by ATP measurements.

It was shown with both test alternatives that the migration of bioavailable organic compounds was elevated within the first weeks of use, followed by a lower but constant level over the 16 weeks investigated.

The migration of bioavailable organic compounds from the material surfaces was influenced by diffusion over the solid-liquid boundary layer under sterile conditions. This caused an inversely proportional relationship between amount of migration expressed per unit surface area of material and ratio between material surface to water volume (S/V-ratio). The thickness of the solid-liquid boundary layer was affected by gentle shaking of the water phase, which increased the migration under sterile conditions. With the presence of an active biomass, which continuously consumed migrating bioavailable compounds, the migration limitation due to diffusion over the solid-liquid boundary layer was significantly reduced. As result neither varying S/V-ratios nor gentle shaking of the water phase affected the amount of migration with the materials tested.

It was shown that the characteristics of the inoculum highly influenced the amount of biomass produced during biotic investigations. Therefore demands to the inoculums' microbial diversity are required to ensure uniform results.

No replacement of the test water versus replacement once a week or once every second week, did not appear to affect the biomass production over 16 weeks of biotic incubation.

Incubation temperatures of 10ºC and 25ºC had no significant effect on the migration of bioavailable compounds measured as biomass production during biotic incubation, though a tendency for higher biomass densities was seen at the lower temperature.

In addition to batch investigations, biofilm formation on polymers can be investigated in continuous flow model systems. Since growth will be supported both by substrate from the water phase and from the material, special requirements apply for flow model systems for use with polymers. A continues flow model system was developed, taking into account different combinations of material contact time and flow velocities. Commercial available pipe was used as exchangeable test pieces for biofilm sampling. Three materials were investigated in separate systems: PVC-C as test material, stainless steel as negative control and PVC-P as positive control.

With the materials used, there was no significant difference between biofilm densities in batch set-ups and in continuous flow model systems over 16 weeks of incubation. During 43 weeks of investigation the biofilm density continuously increased on all three materials, but no significant effect of different combinations of material contact time and flow velocity was observed. A broader range of materials with varying aftergrowth potentials may stress the effect of contact time and flow velocity.

DANISH SUMMARY

Polymere materialer erstatter i stadig højere grad de traditionelt anvendte materialer til drikkevandsforsyning. Polymererne foretrækkes, da de er mere fleksible, mindre modtagelige for brud og har lavere anlægsomkostninger end de traditionelle materialer. Imidlertid kan afgivelse af mikrobielt tilgængelige organiske forbindelser føre til forhøjet bakterieantal i forsyningsnettet. Bakterier vil altid være tilstede i forsyningsnet som plantoniske celler i vandfasen og som biofilm på overflader, men forhøjede bakterieantal er uønskede, da de kan give anledning til driftmæssige, hygiejniske og æstetiske problemer.

Formålet med dette projekt var at øge forståelsen af, hvordan man skal undersøge polymeres eftervækstpotentiale, og derved opnå større indsigt i polymerernes indflydelse på bakterielle niveauer i forsyningsnettet.

Undersøgelserne blev udført ved brug af et abiotisk batch set-up, hvor materialerne blev ekstraheret under sterile forhold, og et biotisk set-up, hvor materialerne blev inkuberet med bakterier tilstede. I den abiotiske test, blev afgivelsen af mikrobielt tilgængelige organiske forbindelser bestemt som AOC_{P17} i vandfasen efter fjernelse af materialet. I den biotiske test blev afgivelsen bestemt som biomasseproduktionen i vandfasen og på materialeoverflader målt som ATP.

Begge test alternativer viste, at afgivelsen af mikrobielt tilgængelige organiske forbindelser var forhøjet de første uger efter ibrugtagning, hvorefter en lavere men konstant afgivelse blev observeret over en testperiode på 16 uger.

Afgivelsen af mikrobielt tilgængelige organiske forbindelser fra materialeoverfladerne var under sterile forhold påvirket af diffusion over væskegrænselaget. Således var der et omvendt proportionelt forhold mellem afgivelse per overfladeenhed af materiale og forholdet mellem materialeoverflade og vandvolumen (S/V-forholdet). Tykkelsen af væskegrænselaget var påvirket af let omrystning vandfasen, hvilket øgede afgivelsen under sterile forhold. Med tilstedeværelsen af en aktiv biomasse, som kontinuerligt omsatte de afgivne forbindelser, fik diffusionen over væskegrænselaget langt mindre betydning. Således var der med de anvendte materialer ikke nogen effekt af S/V-forhold og let omrystning af vandfasen, når bakterier var tilstede.

Det blev demonstreret, at inoculumets karakteristika havde betydning for mængden af produceret biomasse, og der bør således stilles krav til inoculumets mikrobielle diversitet for at sikre overensstemmelse mellem undersøgelser.

Ingen udskiftning af testvandet i forhold til udskiftning en gang om ugen eller en gang hver anden uge havde ingen signifikant effekt på biomasseproduktionen over 16 ugers biotisk inkubation.

Inkuberingstemperaturer på 10ºC eller 25ºC havde ikke signifikant indflydelse på biomasseproduktionen under biotisk inkubation, men der var en tendens til højere niveauer ved den lavere temperatur.

I tillæg til batch set-ups, kan biofilmdannelse på polymerer undersøges i flow model-systemer. Da bakterievæksten vil understøttes af substrat både fra vandfasen og fra materialerne, må der stilles særlige krav til flow model-systemer til undersøgelse af polymerer. Et model-system med kontinuert flow blev udviklet, som tog højde for forskellige kombinationer af kontakttid med materiale og flow-hastighed. Kommercielt tilgængelige rør blev brugt som udskiftelige teststykker til udtagning af biofilmprøver. Tre materialer blev undersøgt i tre separate flow model-systemer: PVC-C som testmateriale, rustfrit stål som negativ kontrol og PVC-P som positiv kontrol.

De undersøgte materialer gav ikke anledning til signifikante forskelle i biofilmdannelse mellem batch-undersøgelser og flow model-systemer i løbet af 16 ugers inkubering. I løbet af 43 uger voksede biofilm-densiteten på alle tre materialer, men der var ikke nogen signifikant effekt af de forskellige kombinationer af kontakttid med materiale og flow-hastighed. En bred vifte af materialer med varierende eftervækstpotentiale vil eventuelt kunne demonstrere en betydning af kontakttid med materiale og flow-hastighed.

CONTENT

1 INTRODUCTION

The materials traditionally used for drinking water distribution, e.g. iron, cast iron, cement and copper, are to an ever increasing extent being replaced by polymeric materials. The polymer types, which have the most widespread use in connection with water distribution, are polyethylene (PE), polyvinylchloride (PVC), polypropylene (PP) and rubber materials for e.g. seals. The polymers are preferred since they are more flexible and less subjected to leakages and corrosion. Also the construction costs for the water supplies are highly reduced with the use of polymers, especially since the flexibility of the polymeric pipe allows it to be pushed inside the exiting pipe line when renewing pipe line. On the negative side, polymeric pipes are not resistant to organic pollution in the surrounding soil, and are more vulnerable towards stones moving due to vibrations in the surrounding soil. Furthermore, bioavailable organic compounds can migrate from the polymers during operation, causing bacterial aftergrowth in the distribution systems.

The purposed of the present project was to enhance the understanding of how to investigate polymers aftergrowth potential, in order to gain better insight into how polymers affect bacterial levels in distribution systems.

The thesis summarises the existing literature and knowledge related to the subject, with detailed description of the experimental work performed given in the papers of the appendixes.

2 BACTERIA IN DRINKING WATER DISTRIBUTION SYSTEMS

Bacteria will always be present in drinking water distribution systems, as planktonic cells in the water phase and as a biofilm on the pipe surfaces. In the biofilm the bacteria appears as sparsely distributed micro colonies encapsulated in a gel-like matrix of extracellular polymeric substances (EPS) consisting of polysaccharides, proteins and lipids excreted by the bacteria (Wingender *et al.*, 1999). The EPS matrix is highly hydrated containing up to 98-99% water (Christensen & Characklis, 1990 cited by Cooksey, 1992) with the EPS components making up 50-90 % or the organic matter (Christensen & Characklis, 1990; Nielsen *et al.*, 1997 cited by Flemming and Wingender, 2001a).

The EPS matrix provides exchange of exoenzymes, cellular debris and genetic material between the bacteria and isolates nutrients from the water phase. Nutrients are continuously lead to the bacteria from the passing water and nutrients tend to accumulate at the solid-liquid interphase (Flemming and Wingender, 2001b; Marshall, 1996), thus attached cells gain access to a larger nutrient load compared to planktonic cells. The EPS matrix furthermore protects the bacteria against environmental changes and most biocides (Blenkinsopp and Costerton, 1991; de Beer *et al.*, 1994; Flemming and Wingender, 2001b; Fletcher, 1992), making attached bacteria more resistance to disinfection (Block *et al.*, 1995; LeChevallier *et al.*, 1988a, 1988b, 1990). The advances of attachment cause biofilm bacteria to outnumber planktonic bacteria in oligotrophic environments such as drinking water distribution systems (e.g. Keevil *et al.*, 1995; van der Wende *et al.*, 1989).

2.1 Aftergrowth and related problems

From the waterworks to the consumers tap, the number of bacteria is likely to increase in the distribution system, a phenomenon entitled aftergrowth. Bacteria in drinking water systems are primary heterotrophic thereby needing C, N and P in an approximate ratio of 100:10:1. In most cases the concentration of Assimilable Organic Carbon (AOC) is the limiting factor for bacterial aftergrowth (van der Kooij *et al.*, 1982). However, in humic-rich waters with high AOC levels (Lehtola, 2002a,b; Miettinen *et al.*, 1997, 1999) or in waters where the phosphorus content is low, e.g. due to removal by chemical coagulation/sedimentation or GAC filtration during the water treatment (Lehtola *et al.*, 2002b; Sathasivan *et al.*, 1998), phosphorus can be limiting (Lehtola *et al.*, 2002a,b; Miettinen *et al.*, 1997, 1999; Sathasivan and Ohgaki, 1999; Sathasivan *et*

al., 1998). When phosphorus is limiting, even small changes in the phosphorus content results in large changes in aftergrowth. Aftergrowth is undesirable, as high bacterial numbers in distribution systems can cause a series of problems.

Biofilms can actively enhance corrosion of metal pipes through microbial induced corrosion (MIC) (Chamberlain, 1992; Emde *et al.*, 1992; Lee *et al.*, 1980). The rough surfaces produced by corrosion products and tubercles function as protected habitat for the bacteria (Allan *et al.*, 1980; Emde *et al.*, 1992; Lee *et al.*, 1980), making MIC a self-perpetuating process.

The aesthetic quality of the water may deteriorate, as high biomass concentrations have shown to cause problems with discolouring, bad taste and odour (e.g. O'Connor, 1975; Mallevialle and Suffet, 1987).

High bacterial numbers can cause a health risk since entering pathogens may be retained and survive in thick biofilms, where they are protected against disinfection (Camper, 1996; Fass *et al.*, 1996; Herson *et al.*, 1991; LeChevallier *et al.*, 1987, 1988a,). It has been shown that elevated coliform numbers exist at AOC levels above 180 µg C/L, while AOC levels below 100 µg C/L supports very low coliform levels (LeChevallier *et al.*, 1992). Since AOC consumes disinfection residuals, the higher the AOC content, the higher free-chorine/chloramines concentrations are needed for disinfection (LeChevallier *et al.,* 1996).

One way to limiting aftergrowth and to enhance disinfection efficiency is therefore to keep a low AOC value throughout the distribution system, i.e. to maintain biostable conditions. Production of biostable water might however prove insufficient to maintain biostable conditions throughout the distribution system, since bioavailable organic compounds can migrate from polymeric materials used in the distribution systems and support microbial growth (Colbourne, 1985; Conroy, 1993; Schoenen, 1989; van der Kooij and Veenendaal, 2001; van der Kooij *et al.*, 1999). One can thus discuss both the aftergrowth potential of the produced drinking water and of the materials used for distribution of the water. Aftergrowth potential is in this context defined as the microbial growth water or material can support.

3 MICROBIAL PARAMETERS FOR ASSESSMENT OF AFTERGROWTH

Numerous methods can be applied in investigations of microbial growth. The most commonly applied microbial parameters in relation to investigating aftergrowth potential and ways to release biofilm samples from the substrata are described below.

3.1 Determination of biomass

3.1.1 Total direct count

Total cell numbers of a sample can be enumerated by direct epifluorescent microscopy after collection of the cells by filtration followed by staining. For total counts the stains Acridine Orange (AO) and 4,6-diamidino-2-phenylindole (DAPI) have the most widespread application. Both stains are DNA stains and bind thus to both active and inactive cells, whereby also non-viable and dead cells are included in the cell count.

3.1.2 Heterotrophic Plate Count

Heterotrophic Plate Count (HPC) is probably the most common enumerating method. Bacteria captured on a solid media are incubated to form colonies visible to the eye. The count depends on the bacteria's ability to grow on the offered media, incubation temperature and incubation time. The closer the incubation conditions mimic the bacteria's natural habitant, the higher percents of the total bacterial number are detectable as colonies. A media with a broad spectrum of substrates in low concentrations, R_2A, is developed especially suitable for enumeration of bacteria from the oligotrophic environment of drinking water (Reasoner and Geldreich, 1985). Prolonged incubation times allow for a larger number of slow growing bacteria to form colonies on the media, which can be advantageous if differences between samples are to be identified. However, even under optimised incubation conditions HPC only provide numbers corresponding to 0.1-10% of the total cell count.

Figure 1: Typical colonies obtained on R_2A ($HPC_{R2A,\ 20^{o}C,\ 14\ days}$) from biostable drinking water produced without chemical treatment and disinfection.

3.1.3 Adenosine TriPhosphate

Adenosine TriPhosphate (ATP) is an important energy carrier in all living cells, and can be used as a measure for cell activity and to some extent for density of microorganisms (Stanley, 1989). The reaction between ATP and D-luciferin is catalysed by firefly luciferase and results in emission of light, which can be measured in a luminometer as relative light units (rlu):

$$ATP + D-luciferin + O_2 \xrightarrow[+Mg^{2+}]{\text{firefly luciferase}} AMP + PP_i + oxyluciferin + CO_2 + light$$

The rlu value is converted to an ATP value by an ATP-standard curve, which is approximately linear over several decades (Figure 2). The characteristics of the sample (e.g. cell density, colour, particles, pH) affect the light emission, wherefore it is necessary to account for the differences between media for preparation of ATP-standard dilutions and the samples by use of an internal standard. Coloured biofilm samples with high particle contents have been seen to reduce the light emission with up to 85%, while 'clean' drinking water samples with low cell density does not affect the light emission when compared to ATP-standard dilutions in tap water (Corfitzen and Albrechtsen, 2004c).

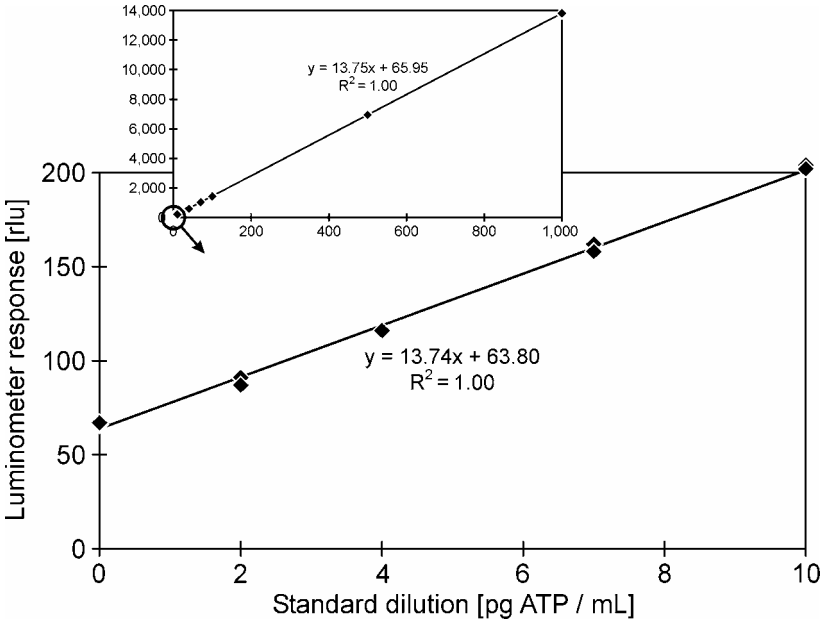

Figure 2: Example of an ATP-standard curve prepared in sterile filtrated tap water measured with Lumin(ATE)/Lumin(EX) reagents from Celsis on an Advance Coupe luminometer (Corfitzen, unpublished data).

The method is advantageous as it has proven very sensitive (detects concentrations of 0.5 ng ATP/L), fast since results are available within minutes, flexible since only small sample volumes (<1 mL) are necessary, and operational since commercial kits and

(semi)automatic luminometers make the measurement procedure simple. The ATP value of a drinking water sample can however not be directly converted to cell numbers, as the ATP content of the single cell depends on strain and growth phase (Stanley, 1989; Lundin, 2000). Extra cellular ATP can contribute significantly to the total ATP value (Corfitzen and Albrechtsen, 2004c), so if only intra cellular ATP is of interest, isolation of the cells by filtration is necessary.

3.2 Assimilable Organic Carbon

The AOC content of a water phase is determined by a bioassay, in which the growth of selected test bacteria, the AOC content in the water can support, is referred to the growth on a reference substrate.

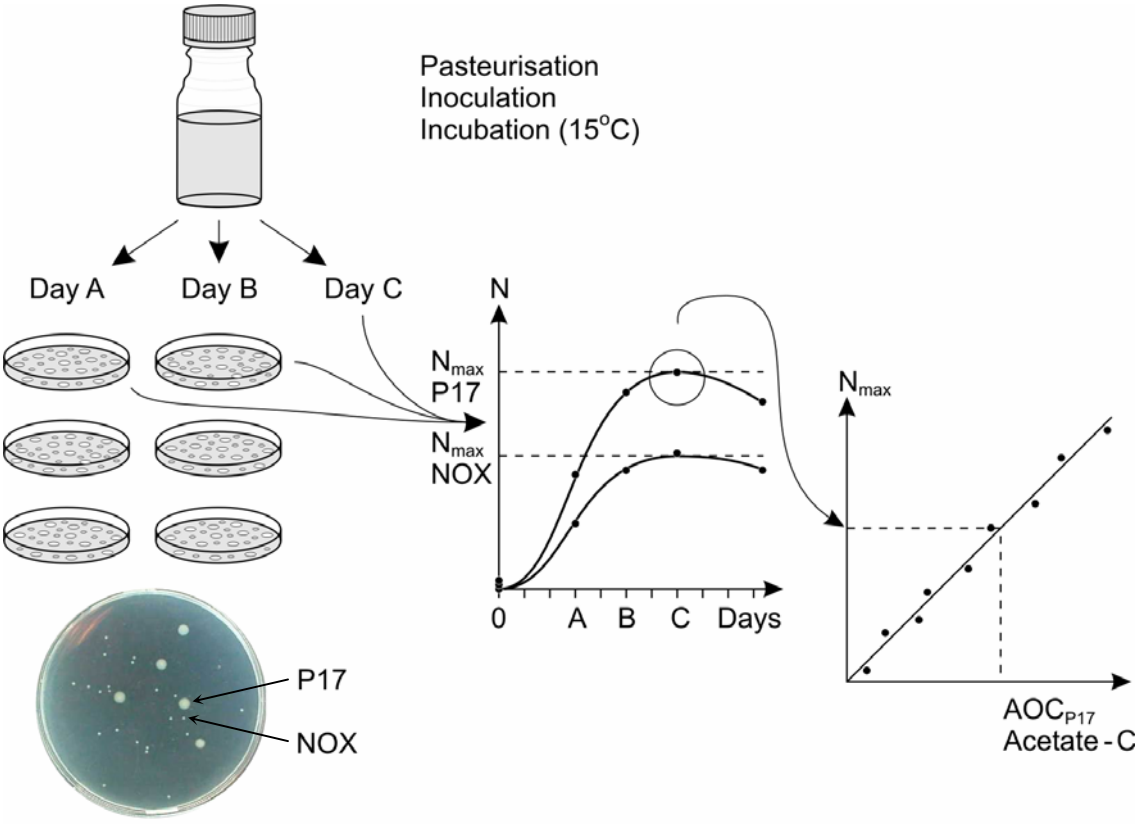

Figure 3: Schematic diagram of the principle of the original AOC method. The two test strains can be separately identified by difference in colony morphology.

The AOC assay is originally developed by van der Kooij *et al.* (1982). After pasteurisation to inactivate the indigenous population, the water sample is inoculated with two pure bacteria strains *Pseudomonas fluorescence* strain P17 and *Aquaspirillum* sp. strain NOX (200-500 CFU/mL) and incubated at 15°C. P17 utilises a wide range of low molecular weight organic compounds while NOX utilises carboxylic acids. The

maximum growth over time (typical periods of 7 to 14 days) is identified with periodical plate counts ($HPC_{R2A,\ 25^oC,3\ days}$). The maximum bacterial numbers for each strain are converted to an AOC value by a yield factor for the two strains established on acetate: 1.2×10^7 CFU NOX/µg acetate-C and 4.1×10^6 CFU P17/µg acetate-C (van der Kooij and Veenendaal, 1995) (Principle sketch in Figure 3).

A variant of the method is developed by LeChevallier *et al.* (1993) in an attempt to reduce the assay time. After pasteurisation, separate samples are inoculated with either P17 or NOX at 1,000 times higher cell concentrations than in the van der Kooij alternative. Bacterial numbers are determined after 2 to 4 days of incubation at 20ºC by ATP measurements. The ATP values are converted to cell numbers by conversion factors between ATP and P17 and NOX, and the cell number is converted to acetate equivalents by the yield factors given by van der Kooij and Veenendaal (1995).

Stanfield and Jago (1989) apply a mixed drinking water population as inoculum instead of pure strains. The indigenous population is removed by filtration, where after the sample is inoculated with a mixed drinking water population and incubated at 22ºC. Daily ATP measurements are used to identify the maximum ATP concentration, which following is converted to an AOC value by a yield factor for the inoculum established for acetate.

Kemmy *et al.* (1989) suggest using a mixture of carbon sources as reference material rather than acetate. The pasteurised sample is inoculated with a mixture of *Pseudomonas flourenscens*, a *Curtobacterium* sp., a *Corynebacterium* sp. and an unidentified coryneform, and incubated at 20ºC for six days. HPC is enumerated by the drop count technique and is converted to an AOC value by a yield factor for the inoculation mixture established for a standard mixture of organic compounds based on peptones and yeast supplemented with acetate and glucose.

ATP measurement demands special equipment end operating skills, which has been limiting the use of the two AOC method alternatives using ATP as parameter for bacterial growth. The simplest approach given by the original method appears to be the one, which has achieved the most widespread extent of application.

3.3 Release of biofilm from materials surfaces

Different approaches are applied to release biofilm from material surfaces for measurement of attached biomass.

Coupons and slides can be transferred to a container with a water phase and vortex vibrated to release the biofilm (e.g. Kerr *et al.*, 1999). The method can be applied for pipe segments with biofilm on the inner surfaces by closing the ends of the pipe segment, filling it with water and vortex vibrate. To increase the release efficiency glass beads can be added to the pipe during vortex vibration (e.g. Zacheus *et al.*, 2000).

Another alternative is to release biofilms by applying sonication to a water phase containing the material sample (e.g. Clark *et al.*, 1994; Corfitzen *et al.*, 2004; van der Kooij and Veenendaal., 1993, 1994, 2001). With low energy sonication multiple treatments are required to release all the biofilm. Three subsequent treatments are sufficient to release biofilm from glass (e.g. van der Kooij and Veenendaal, 1993) while more subsequent treatments are needed for polymers (e.g. van der Kooij and Veenendaal, 1994 and Figure 4).

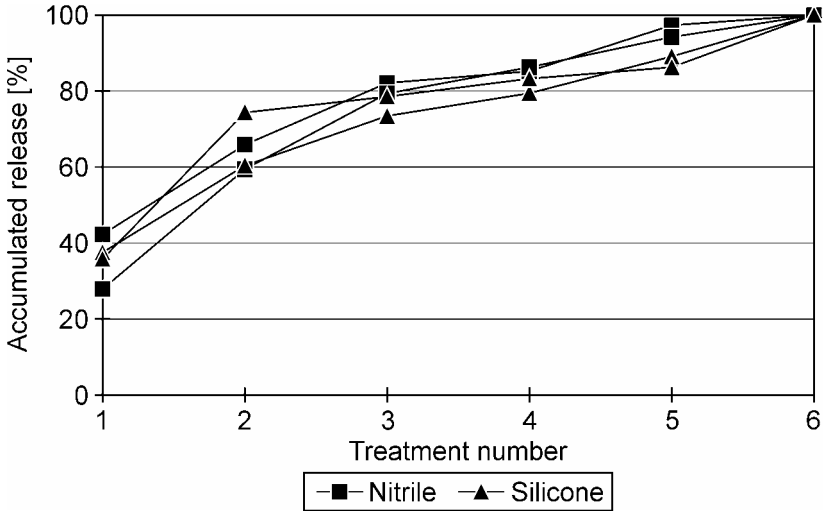

Figure 4: Efficiency of low energy sonication, given as two minutes treatments with a Branson 2210 Sonifier (Branson ultrasonics B.V., Soest, The Netherlands), to release biofilm from polymeric materials demonstrated with nitrile and silicone. Sum of biomass measured as ATP from six subsequent treatments set as 100% (Corfitzen, unpublished data).

Biofilm can also be released from the material surfaces by swapping with for example cotton sticks. The biofilm is following released from the sticks to a water phase by vortex vibration (e.g. Bagh *et al.*, 1999; LeChevallier *et al.*, 1990) or sonication (e.g.; Corfitzen and Albrechtsen, 2004a; Hallam *et al.*, 2001; Veenendaal and van der Kooij, 1999).

4 AFTERGROWTH POTENTIAL OF DRINKING WATER

Distributing biostable water is the first step in ensuring biostable conditions in the distribution system. The treated water's AOC level is a result of the raw water quality and the treatment processes applied at the water work.

4.1 Determining the aftergrowth potential of drinking water

Different approaches can be used to characterise the aftergrowth potential of drinking water. A combination of some of the methods described below will give the most dynamic interpretation of the waters aftergrowth potential.

4.1.1 AOC level

The AOC level of the water can give indication of the water's aftergrowth potential, since the higher the AOC level the higher bacterial growth can potentially be supported. In areas where phosphorus rather than AOC is growth limiting factor, the $AOC_{potential}$ value can be determined after addition of inorganic nutrients (P and N) (e.g. Miettinen *et al.*, 1999). In a study of water samples from 20 water distribution networks distributing drinking water produced from ground water and surface water van der Kooij (1992) establish a guideline for water being biostable at AOC values below 10 µg acetate-C/L. The AOC value enables direct comparison of different water types' biostability.

4.1.2 Growth response tests

A simple way of getting an indication of water's aftergrowth potential is to incubate a water sample and follow the growth of bacteria over time, which AOC from the water can support. Bacterial numbers enumerated by HPC (e.g. Jørgensen *et al.*, 2002) or by ATP (e.g. Corfitzen and Albrechtsen, 2004c) indicate the potential maximum bacterial aftergrowth in the water. Bacterial growth, measured in growth response tests as a measure for biostability, is more complicated than AOC values to compare between water types, since the natural bacterial population and its substrate-need can differ. For comparison of different water types, an inoculum can be added to ensure sufficient microbial diversity in all samples. Biostable water will in general also maintain low ATP values (Figure 5). Van der Kooij (2003) gives as a guideline that water with AOC values below 10 µg acetate-C/L also maintains ATP values below 10 ng ATP/L. This

corresponds with experiences from Danish water supplies, where periodical measurements in three distribution systems, distributing water produced form ground water without chemical treatment or disinfection shows AOC values of 1.5-7 µg acetate-C/L and ATP values below 5 ng ATP/L (Corfitzen, unpublished data).

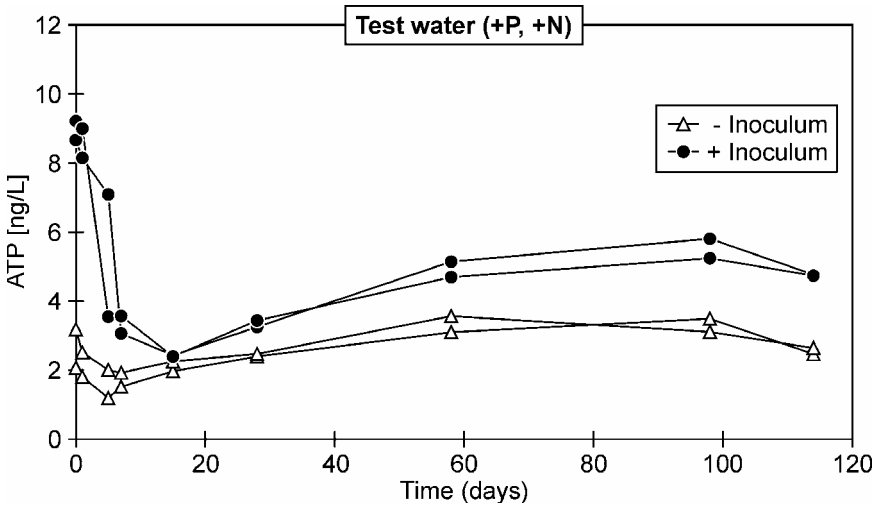

Figure 5: Waters with low AOC values generally also maintain low ATP values during growth response tests. Example of bacterial growth measured as ATP in nutrient amended biostable water with an AOC value of 4.5 µg acetate-C/L. Duplicate water samples incubated at 25ºC with and without addition of a 1% surface water inoculum (Corfitzen and Albrechtsen, 2004c).

4.1.3 Biofilm Formation Potential

The aftergrowth potential of water can also be characterised by the Biofilm Formation Potential (BFP), which gives the biofilm density substrate from the water can support.

The biofilm monitor developed by van der Kooij *et al.* (1995) determines the biofilm formation on glass rings in a continuous water flow. The monitor consists of a vertical glass column (length: 60 cm, internal diameter: 2.5 cm) in which glass ring are placed on top of each other. The column is supplied with water at an empty column flow rate of 0.2 m/s. Periodically rings are removed from the top of the column for determination of biofilm density (van der Kooij and Veenendaal, 1993; van der Kooij *et al.*, 1995). The biofilm is released by ultrasonic treatment and the biomass density is determined by ATP and/or HPC. The Biofilm Formation Rate (BFR) determined as the linear increase in biofilm density over time may also be used to characterise the water. Investigations with the biofilm monitor (van der Kooij and Veenendaal, 1993; van der Kooij *et al.,* 1995; Veenendaal and van der Kooij, 1999) showed BFP values of 24-3,000 pg ATP/cm^2 after exposure periods of 78 to 150 days of unchlorinated waters with AOC values between 2 to 40 µg acetate-C/L, giving BFR values of 0-40 pg ATP/cm^2·day.

An alternative monitor is applied by Hallam *et al.* (2001) who use Biofilm Potential Monitors (BPM) consisting of a Delrina acetyl resin in-line filter holder containing glass beads with a nominal diameter of 3 mm. The BPM is supplied with water at an empty filter flow rate of 0.034 m/s for 21 days, where after biomass is determined by extracting ATP directly from the beads. In water types with TOC values of 1.5 to 3.0 mg C/L and chlorine concentration of 0.03 to 3.1 mg/L, Hallam *et al.* (2001) found BFP values of 2 to 1,167 pg ATP/cm^2, with free chlorine residual of 0.2 mg/L ensuring BFP values below 50 pg ATP/cm^2 (Hallam *et al.*, 2001).

4.2 Producing biostable water

The AOC content of treated water can vary from below 5 µg acetate-C/L in water produced from ground water to several 1,000 µg acetate-C/L in water produced from surface waters. Treatment processes at the water works affect AOC levels as summarized in Table 1.

Oxidation processes cleave larger organic molecules to smaller ones thereby making them bioavailable and thus increasing the AOC level. Ozonation greatly increases the AOC level with typical increases of 100-900% (Table 1). Chlorine, chloramines, and hydrogen peroxide typically increase the AOC level of 20-100% (Table 1). Combination of the oxidative treatments leads to additive increases in AOC level (e.g. Miettinen *et al.*, 1998).

Disinfection by UV radiation does not increase the AOC level of the water (Charnock and Kjønnø, 2000; Hengesbach *et al.*, 1993). Studies have shown that water's aftergrowth potential even decreases after UV radiation (Lehtola *et al.*, 2003; Lund and Hongve, 1994), since the AOC decreases due to changes in the molecular fraction of the organic carbon making it less bioavailable (Lehtola *et al.*, 2003).

Even tight membrane filters, such as nanofiltration membranes, do not necessarily retain the low-molecular-weight compounds that make up the AOC content, and have therefore no considerable effect on the AOC level (Charnock and Kjønnø, 2000; Escobar *et al.*, 2000; LeChevallier *et al.*, 1999).

Coagulation/sedimentation processes reduce the water's AOC level in magnitudes of 10-80% (Table 1). The reduction might to some degree be related to bioactivity during the residence time in the clarification tank (Easton and Jago, 1993).

Biological filtration processes reduce the AOC level of the water, since the AOC is consumed by the active biomass, giving reductions of 20-80% (Table 1), dependent of

the bioavailability of the carbon and the contact time. In GAC filters the AOC is removed both by adsorption to the carbon and by biodegradation if bioactivity in the filter is not limited by chlorination. GAC filtration generally leads to AOC reductions of 50-99% (Table 1).

Through an optimisation of the treatment processes (e.g. sufficient retention time in the biological filters) and the sequence of their application, production of biostable water may be achieved.

Treatment	Effect on AOC level	References
Coagulation/Sedimentation	Decreases	(Charnock and Kjønnø, 2000) (Easton and Jago, 1993) (Huck *et al.*, 1991) (Kemmy *et al.*, 1989) (Lehtola *et al.*, 2002)
Ozonation	Increases	(Easton and Jago, 1993) (Hu *et al.*, 1999) (Huck *et al.*, 1991) (Koffskey and Lykins, 1999) (LeChevallier *et al.*, 1992) (Lehtola *et al.*, 2002b) (Miettinen *et al.*, 1998) (Stanfield and Jago, 1989) (van der Kooij *et al*., 1982, 1989) (Zacheus *et al.*, 2000)
Chlorination/ Chloramination/ Hydrogen peroxide treatment	Increases	(Charnock and Kjønnø, 2000) (Easton and Jago, 1993) (Koffskey and Lykins, 1999) (Lehtola *et al.*, 2002b) (Miettinen *et al.*, 1998) (Stanfield and Jago, 1989)
UV-irradiation	Unchanged/Decreases	(Charnock and Kjønnø, 2000) (Lehtola *et al.*, 2003) (Lund and Hongve, 1994)
Membrane filtration	Unchanged	(Charnock and Kjønnø, 2000) (Escobar *et al.*, 2000) (LeChevallier *et al*., 1999)
Biological filtration/ Slow sand filtration	Decreases	(Easton and Jago, 1993) (Hu *et al.*, 1999) (Kemmy *et al.*, 1989) (Koffskey and Lykins, 1999) (van der Kooij *et al.*, 1982, 1989) (Stansfield and Jago, 1989)
GAC filtration	Decreases	(Easton and Jago, 1993) (Hu *et al.*, 1999) (Huck *et al.*, 1991) (LeChevallier *et al.*, 1993) (Lehtola *et al.*, 2002) (van der Kooij *et al.*, 1982) (Volk and LeChevallier, 2002)

Table 1: General effects of water treatment processes on the AOC level in water.

5 INVESTIGATING GROWTH POTENTIAL OF POLYMERS

The majority of the work concerning polymers aftergrowth potential has been carried out during the last 30 years, and unanswered questions still remain. To date, many countries still do not include microbial testing in their approval scheme for polymers to come in contact with drinking water.

During operation in the drinking water distribution systems compounds will migrate from the polymer surfaces. The migrating compounds are monomers from the polymers themselves, additives and degradation products, but the identification of the specific migration compounds is limited (e.g. Anselme *et al.*, 1985; Brocca *et al.*; 2002; Skjevrak *et al.*, 2002), a work hampered by the composition of the polymers being corporate secrets. Since it is not identified which compounds make up the bioavailable fraction, investigations of polymers aftergrowth potential are performed as bioassays, determining the amount of biomass migration of bioavailable organic compounds from the polymers can support. The exact frames for how the bioassay should be performed to give a true picture of the polymers aftergrowth effect in distribution system are still up for debate.

Establishing a knowledge base about specific polymers' aftergrowth potential and the impact of different factors during material investigation is restricted by the fact, that investigations performed within CEN and other investigating bodies have been kept confidential. Thus relatively few research results is available for the public domain. Also the anonymousness of materials complicates comparison of result from different investigations – e.g. the designation "PVC" covers a wide range of products and material properties, which also differ between manufacturers. The manufacturer and product code is required in order to identify specific products for comparison.

In the following chapters, the present knowledge concerning investigation of polymers' aftergrowth potential, as given by per-reviewed periodicals and similar publications, is summarised.

6 MICROBIAL BATCH TESTING OF POLYMERS

The simplest approach with the longest tradition is to investigate polymers aftergrowth potential in batch test set-ups.

6.1 Established batch test methods

Microbial batch or semi-batch testing of polymers has been applied for the last 25 years, and at least four established methods are used for approval of materials within Europe: The British Mean Dissolved Oxygen Difference (MDOD) test, the German Slime Production (SP) test, the Austrian pipe incubation test and the Dutch Biomass Production Potential (BPP) test. The four methods are briefly described below and summarized in Table 2. In all four methods, demands to the test water or addition of inorganic nutrients ensures that AOC is the growth limiting parameter.

Parameter	MDOD test (BS 6920) Britain	SP test (DVGW W270) Germany	BPP test The Netherlands	Pipe incubation (Önorm B5018) Austria
Duration [weeks]	7	26	16	13
Water replacement	2x/week	Continuous	No	1x/week
Inoculum	River water	-	River water	-
Test water	Dechlorinated tap water	Tap water	Slow sand filtrate	Dechlorinated tap water
Temperature [ºC]	30	Ambient	25	22
Surface (S) of test material [cm^2]	150	800	12×8	1,257
Volume (V) of test water [mL]	1,000 mL	(Continuous)	600 mL	1,257 mL
S/V-ratio [cm^{-1}]	0.15	Not applicable	0.16	1
Microbial activity measure	Oxygen	Slime volume	ATP	ATP/ HPC
Control materials, positive/negative	Paraffin wax /Glass	PVC-P, Paraffin/ Stainless steel	PVC-P/ Glass, Stainless steel	PVC-P/ Glass

Table 2: Summary of four existing methods for microbial batch or semi-batch testing of polymers to come in contact with drinking water.

6.1.1 Mean Dissolved Oxygen Difference test

In Britain the mean dissolved oxygen difference (MDOD) test, first described by Colbourne and Burman (1979), has since the beginning of the 1980s been the standard (BS 6920) for testing of non-metallic materials in contact with potable water. Oxygen consumption in the water phase in the presence of the test material is used as indirect measure of microbial activity. The material is incubated for seven weeks with water exchanges twice a week. The concentration of dissolved oxygen is measured in the water phase after 5, 6 and 7 weeks of incubation. The average difference in dissolved oxygen concentration between test material and test water control for the three measurements defines the material's aftergrowth potential. Validation demands for the test are an oxygen concentration in test water control of 8.5±2.5 mg/L and MDOD of 0.0±0.6 mg/L for negative glass control and 7.5±2.5 mg/L for positive paraffin wax control. Materials with MDOD > 2.3 mg/L are not considered suited for use in connection with drinking water (BS 6920, 2000).

6.1.2 Slime Production test

In Germany the slime production (SP) test, first described by Schoenen and Schöler (1983), have also been used as standard since the 1980s (DVGW W270) for testing of materials in contact with drinking water. The production of slime on test material is used as measure of microbial activity. Sheets of material are incubated in continuously flowing water for 13 weeks. Hereafter the volume of slime produced on the surfaces is scraped off, and the 13-week incubation with following scraping is repeated. The volume of slime scraped at the two occasions defines the material's aftergrowth potential. Validation demands for the test are no growth on the negative control in any of the two test periods and growth on the positive control in both test periods (around 6 mL/800 cm^2 for paraffin and around 15 mL/800 cm^2 for PVC-P). Materials with SP values over 0.1 mL/800 cm^2 during one or both test periods are considered unsuitable for used in contact with drinking water (DVGW, 1998).

6.1.3 Biomass Production Potential test

In the Netherlands the Biofilm formation Potential (BFP) test was first introduced in the 1990s based on biomass measurements only on material surfaces (van der Kooij and Veenendaal, 1994). Later the test was expanded to include biomass measurement in the water phase and termed Biomass Production Potential (BPP) test, where microbial activity is measured as ATP (van der Kooij and Veenendaal, 2001). Pieces of test material are incubated for 16 weeks without water replacement. The development in biomass is followed over time as the sum of ATP in the water phase and on the material

surfaces expressed per surface area of material (biofilm released by sonication). The average of ATP values measured after 8, 12, 16 weeks of incubation defines the materials aftergrowth potential. Values from a material database are use to validate test results. Values range from less than 100 pg ATP/cm^2 for glass and stainless steel used as negative controls to more than 10,000 pg ATP/cm^2 for the PVC-P positive control (van der Kooij *et al.*, 2003b).

6.1.4 Pipe incubation test

Recently a method has been developed in Austria (Önorm B 5018). Microbial activity is measured as HPC in the water phase and as ATP in the biofilm. Pipes of test material are incubated vertically with aeration from below. The water is replaced on a weekly basis and the HPC in the water phase is measured after 1, 2 and 3 months of incubation. After three months the biofilm on the inside of the pipes is measured by ATP (biofilm released by sonication). The values for HPC and ATP together define the material's aftergrowth potential. Validation demands for the test is an at least 100 times higher HPC value in the water phase for the PVC-P positive control than for the glass negative control, and an at least 10 times higher ATP value of the biofilm for the PVC-P positive control than for the glass negative control. Materials with HPC values in the water phase 10 times higher or ATP values of the biofilm five times higher than for the glass negative control are not recommended for use in contact with drinking water (Önorm, 2002).

6.1.5 Comparison of established batch test methods

Though a large number of materials are tested with the established batch test methods each year (e.g. 254 materials were tested in 2000 by the British method at The Water Quality Centre, UK), only few results are made public. Typical levels for a large number of materials in MDOD test are given by Colbourne (1985) and values form preliminary investigations of materials with the SP test are given by Schoenen and Schöler (1985). Van der Kooij and Veenendaal (2001) and Veenendaal and van der Kooij (1999) states values for a limited number of materials in the BPP test, and general levels for materials in the BFP test have been given by van der Kooij *et al.* (1999).

Comparisons between methods with limited numbers of materials have been performed by Schoenen and Colbourne (1987), van der Kooij *et al.* (2003a), and have also been reported in confidential reports within CEN and to the UK Drinking Water Inspectorate. It has been demonstrated that the methods do not give concordant results. In some cases materials ended up on different sides of the pass/fail criteria in the different tests. In other cases, materials ranked similar by two of test methods, but gave a non-linear

relation between the responses of the method-specific activity parameter. A general remark was though, that the sensitivity to differentiate between materials was highest with the periodical ATP measurements applied in BPP method.

The four methods differ substantially in the outlined test conditions such as water replacement scenario, use of inoculum, ratio between material surface and water volume (S/V-ratio) and the biomass activity measure (Table 2). This diversity in test conditions complicates the identification of reasons for the observed deviations. It is however essential to understand the effect of different factors in order not to make wrongful conclusions regarding material effects during use in distribution systems.

6.2 Factors affecting migration during microbial batch testing

Comprehensive knowledge and practical experience with microbial batch test investigation of polymeric materials aftergrowth potential presumably exist at testing laboratories, but only little is published about how different factors affect the results.

The significance of test conditions during microbial batch testing of polymers was investigated by Corfitzen *et al.* (2004) and Corfitzen and Albrechtsen (2004b) using principals given by the BPP test in a biotic set-up. The migration of bioavailable organic compounds (in the following referred to as 'bioavailable migration') was measured as the sum of biomass production in the water phase and on material surfaces determined by ATP. Pieces of material were incubated in inoculated tap water amended with N and P at S/V-ratios of approximately 0.2 cm^{-1} during 16 weeks. Table 3 states the ranking of investigated materials determined as the average of biomass values after 8, 12 and 16 weeks of incubation at 25ºC with weekly water replacements.

Material	BPP [pg ATP/cm^2]
Glass	25±9
Stainless steel	39±12
PVC-C	46±13
PEX	68±22
PE	137±23
Silicone	262±86
Nitrile rubber	3,018±683
PVC-P	9,344±3,456

Table 3: Materials ranked after aftergrowth potential given by the BPP value. Materials were incubated in inoculated tap water amended with P and N at 25ºC during 16 weeks at S/V-ratios of 0.2 cm^{-1} with weekly water replacements. (Corfitzen et al., 2004; Corfitzen, unpublished data)

In addition materials were extracted under sterile conditions (Corfitzen *et al.*, 2004) in an abiotic set-up. After extraction and removal of the material, the migration to the water phase was determined as AOC_{P17} by the van der Kooij method (van der Kooij and Veenendaal, 1995).

6.2.1 Inoculum

Since a wide range of compounds is migrating from the polymers, a broad bacterial diversity is required to ensure utilization of all the bioavailable compounds. Thus the characteristics of the inoculum may significantly influence the biomass production during material investigation. This was observed in the biotic set-up when incubating samples with nitrile rubber at two occasions. The 1st and 2nd set of samples were inoculated with inoculum from the same source (Lake Arresoe, DK), but the 2nd set resulted in a much higher biomass production, than the 1st set. After 194 days of incubation each of the replicates from the 1st set of samples was divided into two sub-samples, and half of them were re-inoculated with water phase from the 2nd set. During two weeks of continued incubation the biomass concentration in the re-inoculated sub-samples increased significantly (130 times the original concentration measured as ATP), without any similar increase in the original samples (Figure 6). The inoculum for the 1st set of samples was collected at a much lower water temperature, than the inoculum for the 2nd set, and may therefore have contained a less diverse bacterial population. (Corfitzen, unpublished data). It is thus required to have specific demands for the bacterial characteristics of the inoculum in order to guarantee consistency in results between investigations.

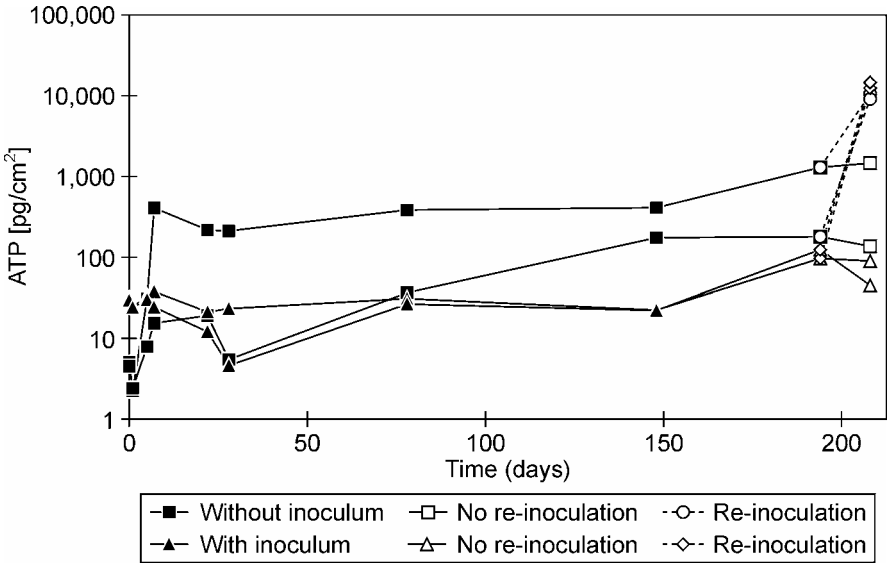

Figure 6: Bacterial growth measured on nitrile rubber as the sum of ATP in water phase and on material surfaces. After 194 days of incubation, with no water replacement at 25°C in nutrient amended test water with and without addition of inoculum, this first set of samples were divided into two sub-samples. Half of the sub-samples were re-inoculated with water phase from a later started set of nitrile rubber samples, which had resulted in higher biomass concentration. The two sets of samples were inoculated with inoculum from the same source, but collected at different water temperature (Corfitzen, unpublished data).

6.2.2 Development in migration over time - effect of water replacement

To elucidate the effect of water replacement, investigations with four polymeric materials and glass were performed in the biotic set-up with no replacement of the water versus replacement once a week or once every second week. The results showed that the main NVOC migration from the polymers occurred within the first six weeks of incubation, where after no significant further migration of NVOC was observed (Corfitzen and Albrechtsen, 2004b).

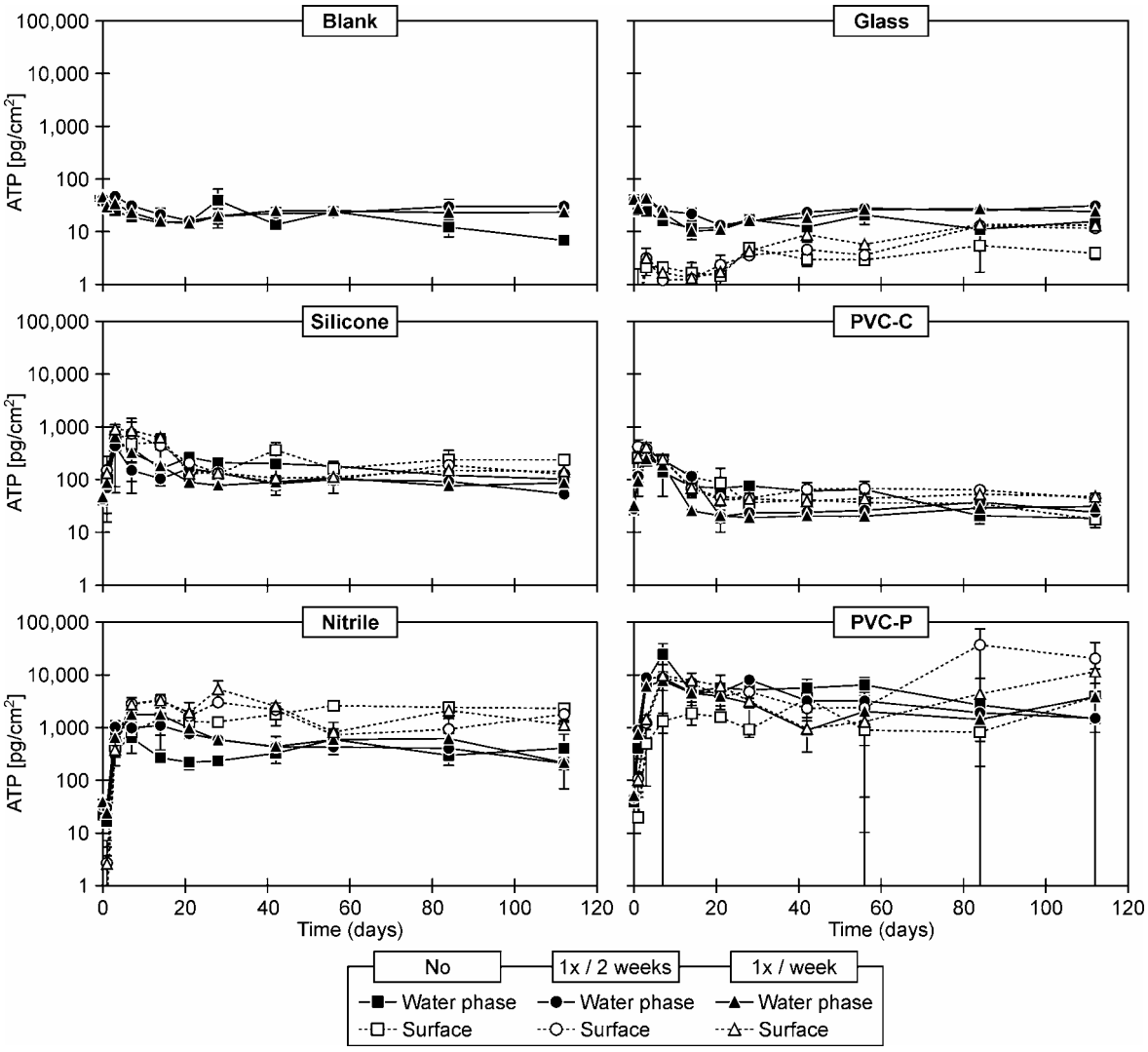

Figure 7: Effect of no water replacement versus replacement once a week or once every second week on biomass production during incubation of polymers. Material pieces were incubated under static conditions at 25 °C at S/V-ratios of approximately 0.2 cm^{-1}. Biomass on material surfaces and in the water phase was measured as ATP during 16 weeks of incubation. Error bars give the standard deviation between duplicate samples. (Corfitzen and Albrechtsen, 2004b)

In contrast, the migration of bioavailable organic compounds from the materials did not level off during the 16 weeks tested. The bioavailable migration peaked within the first two weeks of incubation, but after three weeks a lower but relative constant level was maintained throughout the remaining of the period tested (Figure 7) (Corfitzen and Albrechtsen, 2004b). The same pattern was observed over four sequential sterile extractions of 15 days of a PE material, where the first extraction showed a slightly higher bioavailable migration than the three following extracts, which had similar values (Corfitzen *et al.*, 2004). This indicated that some surface bound compounds was easily flushed off, followed by a constant bioavailable migration from the materials.

Although the biomass in the water phase made up a significant part of the total biomass (up to 50% during the constant level migration – up to 80% for glass) there was no consequent effect on total biomass production as a result of no replacement of the water versus replacement once a week or once every second week, neither measured as ATP (Figure 7), nor as $HPC_{R2A,\ 20^{o}C,\ 28\ days}$ (Corfitzen and Albrechtsen, 2004b). Despite these results, some degree of periodical water replacement could be argued for, since build-up of potentially toxic concentrations of non-bioavailable migration compounds is avoided by periodical water replacements, and well as the risk of nutrient depletion.

6.2.3 Migration kinetics

If the bioavailable migration from the polymer surfaces is controlled by diffusion processes in the solid-liquid boundary layer, the flux from the material will be given by:

$$N = k_w \times (C_{eq} - C_w)$$

- N: Flux (migration)
- k_w: Mass transfer coefficient in boundary layer
- C_{eq}: Equilibrium concentration in water phase
- C_w: Actual concentration in water phase

Thus the lower the actual water phase concentration, the higher driving force for the migration (Figure 8A). At higher S/V-ratios the water phase concentration will be higher, which will limit the migration over the solid-liquid boundary layer expressed per unit surface area of the polymer.

The amount of migration will then furthermore be controlled by the thickness of the solid-liquid boundary layer. Gently shaking of the water phase will decrease the thickness of the boundary layer thereby increasing the amount of bioavailable migration.

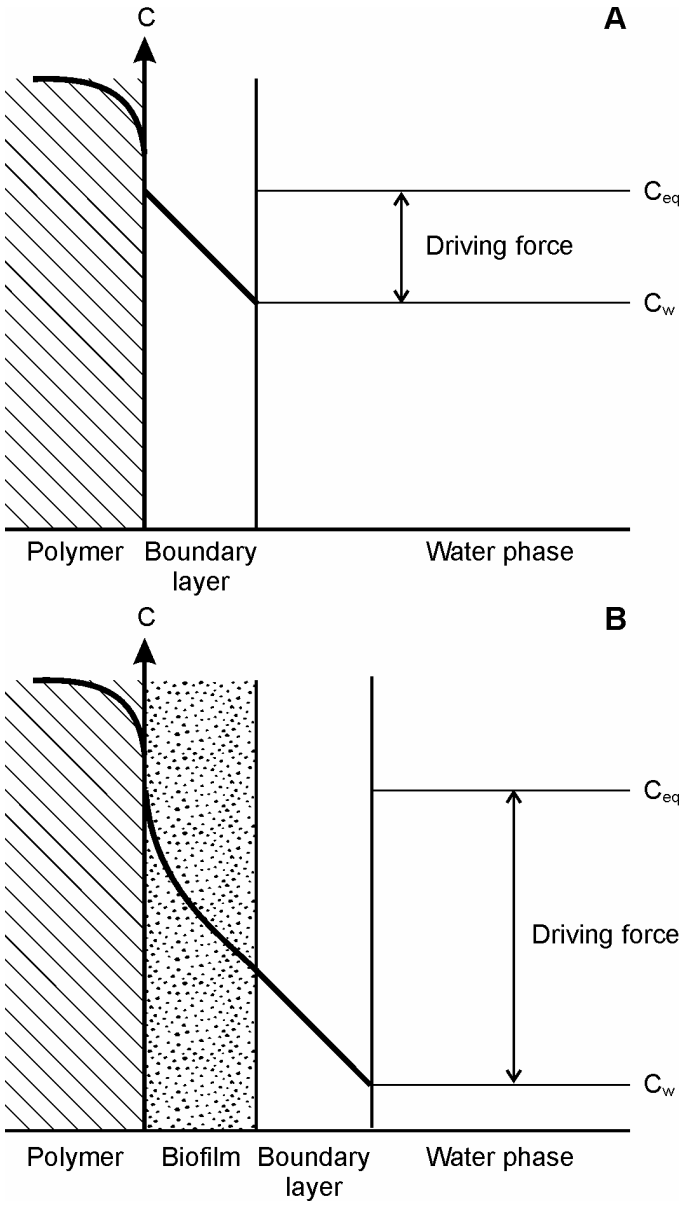

Figure 8: Migration of bioavailable organic compounds from polymer surfaces if influenced by diffusion in the solid-liquid boundary layer. A: Under sterile conditions the driving force for the migration will be given by the difference between the equilibrium concentration in the water phase (C_{eq}) and the actual water phase concentration (C_w). B: When bacteria are present, the continuous consumption of migration compounds in the biofilm will increases the driving force for the migration. (Corfitzen et al., 2004)

Sterile extractions of PE materials in the abiotic set-up supported the hypothesis that bioavailable migration from polymer surfaces is controlled by diffusion over the liquid-solid boundary layer (Corfitzen *et al.*, 2004). Gentle shaking of the incubation containers during extraction resulted in 20-30% higher bioavailable migration than during static extraction. Sterile extraction of two PE materials at varying S/V-ratios (0.07-1.38 cm^{-1}, corresponding to inner pipe diameters of 57.1-2.9 cm) showed an

inverse proportional relationship between the amount of bioavailable migration expressed per unit surface area, and S/V-ratio (Figure 9), thus indicating diffusion-limitation over the boundary layer at higher S/V-ratios.

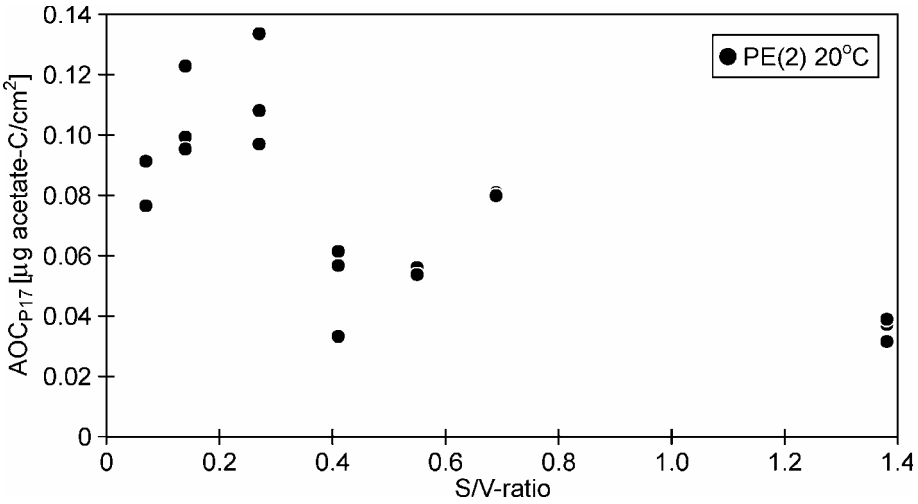

Figure 9: Effect of S/V-ratio on the AOC_{P17} migration from a PE material during sterile extraction, expressed per unit surface area of material. The material was extracted at 20℃ during 15 day s under sterile, non-static conditions, at seven S/V-ratios. Replicates are subtracted arithmetic mean of blanks on water alone. (Corfitzen et al., 2004)

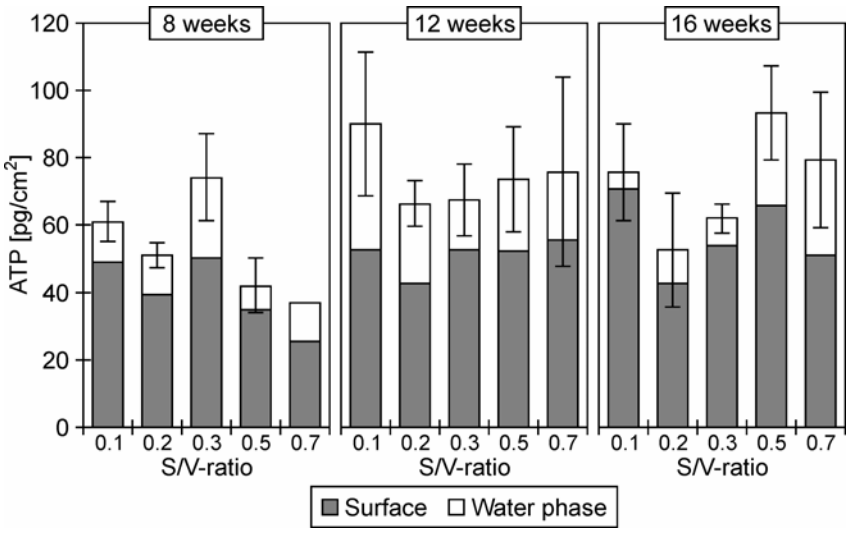

Figure 10: Effect of S/V-ratio on the bioavailable migration from a PE material in the presence of bacteria. Materials were incubated in inoculated test water at 20℃ at five S/V-ratios during 16 weeks with weekly water replacement under static conditions. The replicates are subtracted the arithmetic means of blanks on water alone. Error bars give the standard deviations of duplicate samples. (Corfitzen et al., 2004)

When an active biomass is present during incubation of materials, a biofilm is formed on the material surfaces, which might consume the migrating bioavailable compounds more or less continuously. Thus the water phase concentration will be lowered and the driving force for the migration increased (Figure 8B). Continuously consumption of bioavailable migration by the bacteria in the water phase will likewise increase the driving force. Thus diffusion-limitation will be significantly reduced, enhancing the amount of bioavailable migration. A continuous consumption of bioavailable migration might reduce the significance of the solid-liquid boundary layer, so the effect of gentle shaking of the water phase will be reduced.

Reduction of the diffusion-limitation over the boundary layer in the presence of bacteria was demonstrated with a PE material in the biotic set-up (Corfitzen *et al.*, 2004). Incubation of material in the presence of bacteria at five S/V-ratios (0.1-0.7 cm^{-1}, corresponding to inner pipe diameters of 40.0-5.7 cm) and with weekly water replacements showed no consistent difference in biomass production, between S/V-ratios (Figure 10). Incubation of the material at two S/V-ratios with and without gentle shaking of the incubation containers gave similar levels of biomass production. The growth potential of the applied PE material was relatively low, why the effect of bacteria overruling diffusion-limitation over the solid-liquid boundary layer might be less pronounced for materials with higher growth potential.

6.2.4 Effect of temperature

Glass, steel and three polymeric materials were investigated in the presence of bacteria at 10ºC and 25ºC in the biotic set-up with weekly water replacements. There was no significant difference in biomass production measured as ATP and $HPC_{R2A,\ 20ºC,\ 28\ days}$ (Figure 11). However, there was a general tendency for higher biomass concentrations at lower temperature, which disagrees with the common assumption that migration increases with temperature. For one of the tested materials (a PE material), the temperature dependency was opposite, which indicated that the temperature dependency of migration might vary between materials (Corfitzen and Albrechtsen, 2004b).

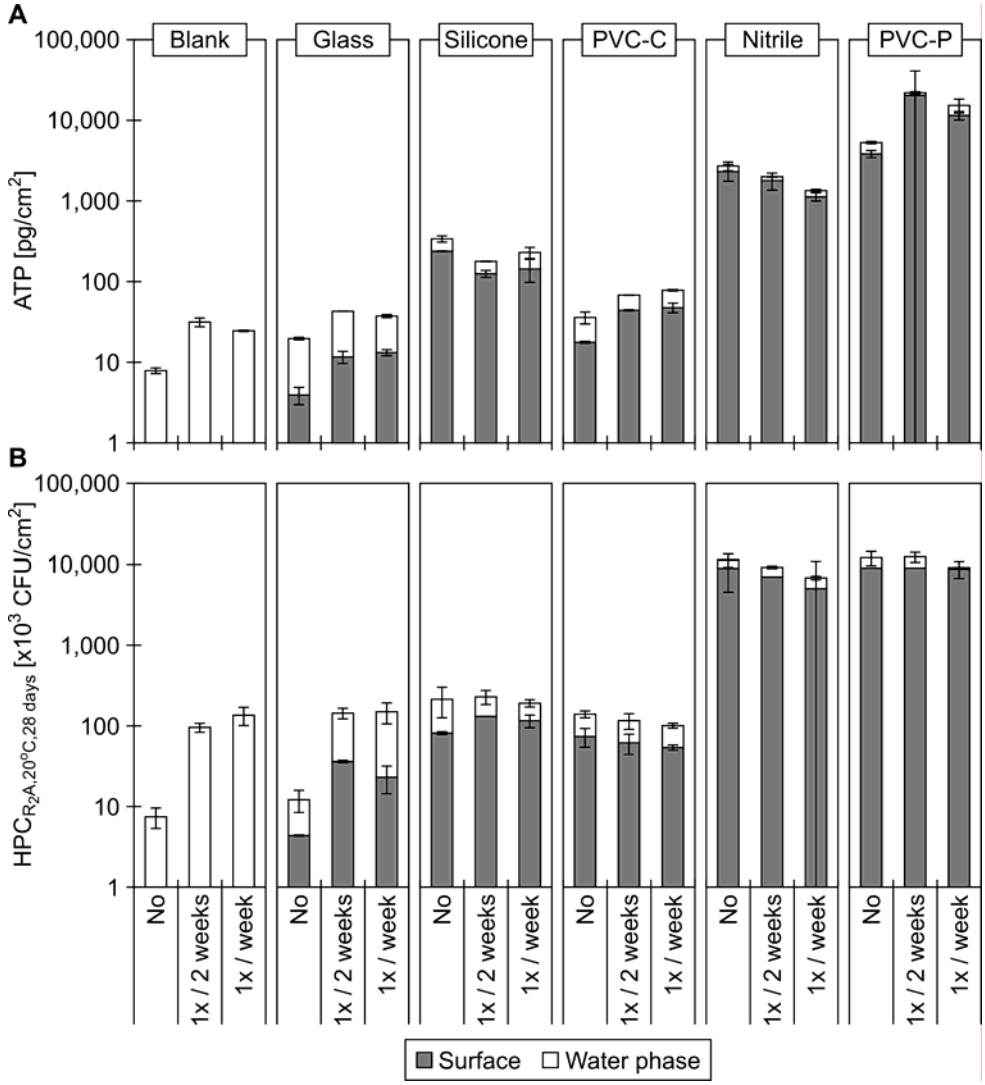

Figure 11: Effect of temperature on biomass production during 16 weeks incubation of polymers. Sample pieces were incubated in inoculated test water under static conditions at S/V ratios of 0.17 cm^{-1} with weekly water replacement at either 10 °C or 25 °C. Biomass was measured on surfaces and in the water phase as ATP after 8, 12 and 16 weeks of incubation, and as $HPC_{R2A,20°C,28\ days}$ after 16 weeks of incubation. Error bars give standard deviation for duplicate samples. (Corfitzen and Albrechtsen, 2004b)

7 BIOFILM FORMATION ON POLYMERS IN FLOW MODEL SYSTEMS

Even an optimised method for microbial batch testing of polymers might not give a true estimate of the polymers aftergrowth potential during operation in drinking water distribution systems. Biofilm formation in flow systems is influenced by both the substrate loading and the shear stress (flow velocity) (van Loosdrecht *et al.*, 1995; Stoodley *et al.*, 1999), so continuous flow models systems simulating the operation conditions in distribution systems may provide a better estimate.

7.1 Flow model systems used for investigating biofilm formation

Different approaches toward establishing flow model systems for investigation of biofilm formation have been made. Some general descriptions of different system principles and systems examples are given in the following.

Flow incubation

A water flow passing small coupons or pieces of test material situated inside an incubation device (including the Robbins device (McCoy *et al.*, 1981) and modification of this (e.g. Manz *et al.*, 1993; Jass *et al.*, 1995; Schwartz *et al.*, 1998; Kerr *et al.*, 1999)) is the simplest form of a flow model system. Coupons or test pieces can either be inserted in the water stream (e.g. Hallam *et al.*, 2001; Niquette *et al.*, 2000; Pedersen 1986; Zacheus *et al.*, 2000) or flush mounted along side of the incubation container, for periodical biofilm sampling. The residence time and the flow velocity in such flow through systems only depend of the inlet flow rate, often resulting in high flow velocities and short material contact time.

Flow-through-pipe systems

Flow-through-pipe systems with the water flow passing inside pipe sections have also been applied for investigating biofilm formation. Hallam *et al.* (2001) closed ends of pipe sections (45 cm long with a diameter of 100 mm) with PVC plates and applied flow through valves in the plates at a flow velocity of 0.002 m/s, to give a contact time with each pipe section of 3.5 minutes. After a given test period, biofilm were sampled by swapping at the outlet end. Zacheus *et al.* (2000) applied flow through a line of 11 connected pipe segments (each 10 cm long with a diameter of 10 mm), allowing for periodically harvesting of pipe segments for biofilm sampling. The water was fed at a flow velocity of 2.3×10^{-4} m/s, which resulted in a material contact time with each pipe segment of 7.1 min. LeChevallier *et al.* (1990) connected pipe sections (each 30.5 cm

long and 1.27 cm in diameter) altering 14 times between 4 materials (21.95 meters of total pipe line). Connections made by quick-fit couplings allowed for periodical biofilm sampling. Water flow was applied to a flow velocity of 0.066 m/s, giving a material contact time with each pipe segment of 4.6 seconds. Delanoue *et al.* (1997) had continuous flow through a 1.3 km long pipe. After 500, 900 and 1,300 meters sampling stations were inserted consisting of pipe segments (50 cm long with a diameter of 10 cm) of seven materials inserted as part of the pipe line. Total residence time at the three sampling stations were 13, 31 and 50 hours respectively, but with a material contact time with each sample pipe segment of 50 seconds at a flow velocity of 0.01 m/s. Veenendaal and van der Kooij (1999) simulated the effect of domestic tap usage patterns on biofilm formation on polymeric materials. Pipe lengths of polymer material making up a total length of 5 m had exchangeable pipe segments for biofilm sampling inserted at the outlet end. Water was tapped in intervals of around 30 seconds between stagnation periods of 0.5–8 hours. During tapping the flow was approximately 260 L/h, corresponding to 0.4-0.8 m/s depending on the pipe diameters used, resulting in 130 L of water lead through the pipes per day. The flow velocity and residence time in flow-through-pipe systems are solely dependent on the applied flow rate.

Reactor systems

Reactor systems such as the Propella®reactor and the continuous stirred annual reactor (RotoTorque) have been applied in biofilm formation investigations. The continuous stirred annual reactor (RotoTorque) consists of a stationary outer cylinder with removable/exchangeable slides (12-20 pieces) flush-mounted on the inside for biofilm sampling. The speed of an inner rotation drum establishes and controls the shear stress at the slide surfaces at the outer wall and the residence time is controlled by the inlet flow. Typically the annual reactor contains water volumes of 0.5-1.5 litre with flow velocities corresponding to 0.3-0.9 m/s and residence times of 0.5-2 hours (Camper *et al.*, 1996, 2003; Gagnon and Huck, 2001; van der Wende *et al.*, 1989). The Propella®reactor is constructed of a pipe segment with a cylinder inserted. A propeller inside the inner cylinder establishes and controls the flow between the cylinder and the pipe wall. The retention time is controlled by the inlet flow. Test coupons for biofilm sampling can be inserted in the outer pipe wall flush-mounted with the surface of the wall. Typically the reactor consists of a 10 cm diameter and 50 cm long outer pipe and is operated at a flow velocity along the pipe wall of 0.2 m/s and a residence time of 24 hours (Holt *et al.*, 1998; Appenzeller *et al.*, 2001). In both reactor types is the inlet flow rate independent of the flow velocity.

Loop systems

Loop systems, dividing the flow model system into section, have also been applied for investigations of biofilm formation. A loop system, where each loop in the system consisted of a 31 meter long and 100 mm diameter cement lined cast iron pipe with a volume of 24 litres, has been applied by Block *et al*. (1993, 1994, 1995) and Clark *et al.* (1994). 21 cement test coupons of 2 cm^2 were flush-mounted with the inner pipe wall. A continuous flow of 10 l/h gave a flow velocity along the pipe wall of 1 m/s and a residence time of 24 hours. Another loop system applying recirculation was constructed by Boe-Hansen *et al.* (2002). Each loop in the system consisted of 12 meter long and 50 mm wide squared stainless steel pipe with a volume of 21 litres. 70 test coupons of 7 cm^2 were flush-mounted with the inner pipe wall. Recirculation gave a flow velocity at the pipe surface of 0.07 m/s independent of the continuous flow rate resulting in a residence time of one hour.

7.1.1 Flow model system investigations involving polymers

Polymers have been applied as biofilm substrata in flow model systems, but in most cases the aim has been to investigate biofilm formation caused by the waters substrate content, with inert substrata such as stainless steel, cement or non-growth promoting PVC. In other investigations the aim has been to investigate the effect of disinfection (e.g. ozone concentration and chlorination dose), where polymers have been used together with for example cement or stainless steel to represent different substrata choices. Investigations of biofilm formation in flow model systems involving polymers are summarised in Table 4. Generally, the investigations has concluded that iron products resulted in the highest biofilm densities due to the large area of corroded surfaces, and also possessed the highest disinfection demand. Cement ranked high in biofilm density in some of the investigations while low in others. Lowest biofilm densities were found on stainless steel and glass, while the remaining tested materials ranked in between in no general order, which most likely is cause by different (e.g. PVC and PE) materials tested in the different investigations. Overall, polymers supported microbial growth, but with the materials applied, organic content and disinfection residual in the water had greater significance on the biofilm formation than the material choice. One noteworthy conclusion was that the biofilm densities found in the pipe flow system simulating tap patterns by Veenendaal and van der Kooij (1999) were lower than biofilm densities for the same materials in the BFP test.

Thus very few investigations have been performed in flow model systems with the primary aim to investigate biofilm formation on polymers to identify their aftergrowth potential, and the flow model systems have not been evaluated for this purpose.

Reference	System	Materials	Disinfection	Goal of investigation
(Pedersen, 1986)	Flow incubation	Glass, Copper, PEMD, PEX, PVC	+	Study if different materials resulted in different biofilm density (biofilm age: 1 and 5 weeks)
(Pedersen, 1990)	Flow incubation	Stainless steel with 3 surface qualities, PVC	+	Study biofilm formation on non-biostimulating materials and effect of surface characteristics (biofilm age: up to 167 days (~24 weeks))
(Zacheus *et al.,* 2000)	Flow incubation	Stainless steel, PVC	-	Study effect of ozone on biofilm formation (biofilm age: up to 19 weeks)
(Niquette *et al.*, 2000)	Flow incubation	Cement linings, PVC, PE, iron, tarred steel, grey iron	+	To study differences in biofilm density on different materials (biofilm age:1 month)
(Hallam *et al.*, 2001)	Flow incubation	PEMD, PVC, cement, glass	+	Study factors (chlorine concentration, material) affecting biofilm growth in distribution systems (biofilm age: 3 weeks)
(Schwatz *et al.*, 1998)	Modified Robbins device (Flow incubation)	PEHD, PVC, steel, copper	+/-	Study genetically diversity and metabolic activity of natural formed biofilms (biofilm age: 2 weeks)
(Kerr *et al.*, 1999)	Modified Robbins device (Flow incubation)	Cast iron, PEMD, uPVC	+	Study biofilm formation and its heterotrophic bacteria diversity between different materials (biofilm age: 3 weeks and up to 10 months)
Delanoue *et al.*, 1997)	Flow-through-pipe system (1.3 km)	Cement lined, bitumen lined PEMD, epoxy, iron	+	Study deposits and biofilm numbers on different materials at three distances from water work applying three different water types (biofilm age: 9-13 months)

Table continues next page

Table continued from previous page

(LeChevallier *et al.,* 1990)	Flow-through-pipe system	Iron, PVC, galvanized iron, copper,	+	Study the efficiency of chlorine and chloramines for controlling biofilm growth (biofilm age: 2 weeks)
(Veenendaal and van der Kooij, 1999)	Flow-through-pipe system	Stainless steel, copper, PEX , PVC-C, PP, Al/PEMD	-	Study biofilm formation between different materials under domestic tap patters (biofilm age: up to 20 weeks)
(Zacheus *et al.,* 2000)	Flow-through-pipe system	PVC, PE	-	Study effect of ozone on biofilm formation (biofilm age: up to 23 weeks)
(Hallam *et al.*, 2001)	Flow-through-pipe system	PEMD, PVC, cement,	+	Study factors (chlorine concentration, material) affecting biofilm growth in distribution systems (biofilm age: 3 weeks)
(Holt *et al.*, 1998)	Propella®	Ductile iron, grey iron, uPVC, PEMD	+	Study disinfection demand of water and materials (biofilm age: up to 3 weeks)
(Camper *et al.*, 2003)	Annual reactor	Epoxy, ductile iron, cement, PVC	-/+	Study the interactions between materials, organics and disinfection and their effects on regrowth (biofilm age: up to 3-4 months)
(Block *et al.*,1993)	Loop system	PVC, cement	+	Study biofilm formation characteristics as function of distance from water work (biofilm age: up to 1 moth)
(Clark *et al.*, 1994)	Loop system	PVC, PE, cement	+	Studying the effect of ozone, chlorination and chloramination (biofilm age: 3-5 weeks)
(Block *et al.*, 1995)	Loop system	PVC, cement	+	Study efficiency of chlorine and chloramine as post disinfection (biofilm age: up to 2 month)

Table 4: Examples of flow model system investigations involving polymers. +:performed with water containing a disinfection residual, -: performed with water without a disinfection residual.

A range of systems for studies of biofilm growth in drinking water, including some of the above described, have been evaluated by (Boe-Hansen *et al.*, 2003) with focus on growth supported only by the water's substrate content. When investigating biofilm formation on polymers, growth will also be supported by the bioavailable migration from the materials, and additional factors have to be considered when evaluating flow model systems, which is summarised in the following paragraph.

7.2 Optimising flow model systems for polymer investigations

To account for a possible effect of water phase concentration of migrating compounds and of possible elevated biomass in the water phase is important that both S/V-ratio and contact time with material are realistic in flow model systems intended for investigating biofilm formation on polymers. Thus the contact time of a few seconds or minutes in some of the described model systems (especially the flow incubation systems) will not reflect realistic conditions. Use of test coupons will in most cases result in unrealistically low S/V-ratios, e.g. in the two described loop systems, the use of polymeric test coupons results in S/V-ratios (between polymeric materials and the water phase) corresponding to pipe diameters of 1.7 meters and 228.5 meters.

The use of coupons and other special-made test pieces poses the risk of making erroneous conclusion regarding the migration, since differences in extruding processes may result in different specific compounds migrate from the final product. It is thus advisable to use commercial pipe segments as test pieces in flow models. The use of submerged pipe segment raises questions regarding how cut surfaces affect the results, since they may allow for migration of compounds that normally not would make it to the surfaces. Another question is how to handle products, which have inner and outer surfaces of different materials.

To avoid interaction between various materials, each material ought to be investigated separately in independent systems, and thus the physical extent (size) of the model system can be limited by practical or financial considerations.

Batch investigation have shown that a constant biofilm density takes several months to establish, which makes it important to perform investigations over long periods of time, and thus will exchangeable test pieces be advantageous.

In order to create realistic flow velocities at the material surface, the flow velocity and residence time need to be independently controlled in most flow model systems. In all the above-mentioned flow model systems only a single flow velocity can be applied at a time, but since it is difficult to predict the effect of different combinations of flow

velocity and growth potentials, it would be advantageous to be able to operate with different flow velocity simultaneously within separate parts of the system.

Based on the outlined criteria, all the flow model systems described in paragraph 7.1 have certain drawbacks in regards to polymer investigations.

7.3 Development of an optimised continuous flow model system

A continuous flow model system was developed (Corfitzen and Albrechtsen, 2004a) optimised for investigations of biofilm formation on polymers. The model system was divided into three main sections (Figure 12):

1. An inlet line – simulating relative short contact times with material (hour magnitude), resulting in a potentially low concentration of migrating compounds in the water phase at a very low flow velocity.
2. A recirculation section – simulating longer contact times with material (day magnitude), resulting in potentially higher concentration of migrating compounds in the water phase at a dynamic flow velocity.
3. An outlet line – simulating longer contact times with material (day magnitude), thus with a potentially higher concentration of migrating compounds in the water phase at very low flow velocities.

Commercial pipe segments were used as test pieces for biofilm sampling. Test pieces were inserted as part of the pipe line, thus only having inner surfaces exposed to the water phase, in each section of the model system. Additional, test pieces submerged in the water stream was inserted in the inlet line. Test pieces were exchangeable by replacement of the ferrules in the connecting unions with O-seals. Separate systems were constructed for each material investigated, with each system constructed in stainless steel (grade 316) together with the material to be investigated.

Three parallel continuous flow model systems were operated with either PVC-C (inner diameter 15 mm) as test material, PVC-P (inner diameter 12 mm) as positive control or stainless steel (inner diameter 16 mm) as negative control. Each model system was fed water from the municipal water supply at a flow rate of 0.7 mL/min giving a flow velocity along the submerged test pieces in the inlet line of 0.16-0.42 cm/min depending on pipe diameter and a contact time around the test pieces of 70 minutes with S/V-ratios of approximately 2 cm^{-1}. The flow velocity along the test pieces with only inner surfaces exposed to the water phase in the inlet and outlet line was 0.35-0.62 cm/min with a contact time for each test piece of 22-28 minutes and S/V-ratios between polymer surface and water phase of 2.5-3.3 cm^{-1} depending on pipe diameter. The recirculation section was constructed partly of stainless steel and partly of the material to be

investigated, to give a S/V-ratio between polymer surface and water phase of 1 cm^{-1} and a total volume of one litre, resulting in a contact time of one day.

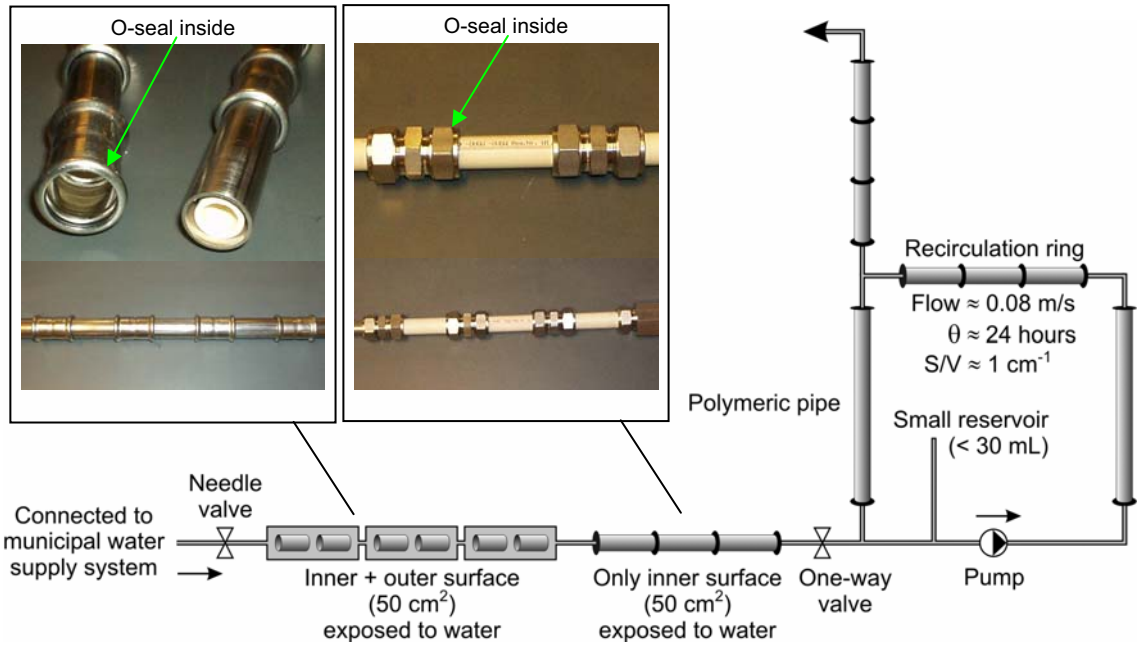

Figure 12: Schematic figure of the continuous flow model system optimised for investigating biofilm formation on polymers.

Biofilm formation

The flow model systems were operated for 43 weeks. Comparison of biofilm formation on test pieces from the inlet line (submerged test pieces and test pieces with only inner surfaces exposed to the water phase) and from the batch test investigations referred in paragraph 6.2.4 showed good agreement between the biofilm densities during 16 weeks of incubation (Figure 13). PVC-C showed same biofilm densities as the negative control stainless steel, while densities for the positive control PVC-P were100 times higher. Thus within the first 16 weeks of incubation there was no significant effect of investigating biofilm formation in the flow model systems over batch investigations, and cut surfaces did not seem to have effect for the materials investigated.

The biofilm densities on test pieces only exposing inner surfaces to the water phase were increasing over the 43 weeks investigated in all three sections of the flow model systems (Figure 14). Again similar biofilm densities were measured for PVC-C and the negative control stainless steel, while levels a factor 100 higher were measured on the positive control PVC-P. For the three materials investigated, flow velocity and contact time did not have any consistent effect on the biofilm formation.

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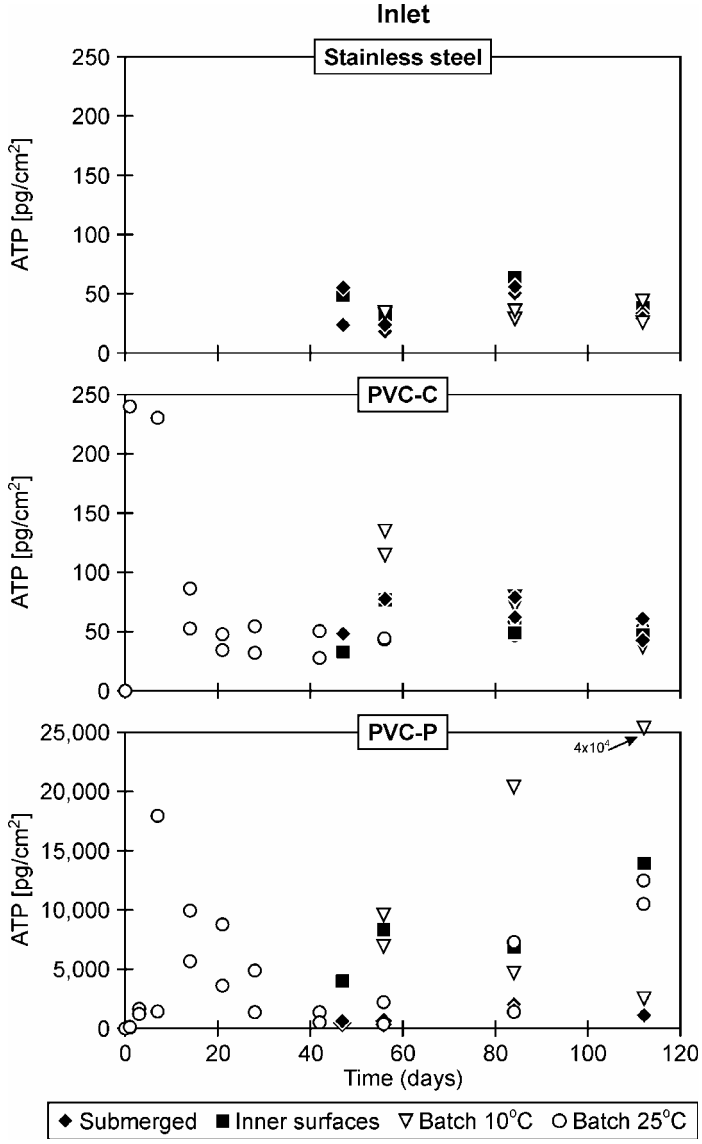

Figure 13: Biofilm formation expressed as ATP on material surfaces in batch set-up (duplicates) or in the inlet line of the continues flow model system as submerged pipe segments (duplicates) or as pipe segments inserted with only inner surfaces exposed to the water phase (single replicates). Investigations performed at 10 ºC; batch test investigation also at 25ºC.

Since the PVC-C material gave rise to similar low biofilm densities as the negative control (stainless steel) and the positive control (PVC-P) gave rise to extreme biofilm densities with large deviations on the determinations, no general conclusion of the effect of flow velocities and material contact time could be made from the preliminary investigations with the continuous flow model system. A broader range of materials with varying aftergrowth potentials may stress the effect of the varying flow conditions and contact times in the three sections of the flow model system

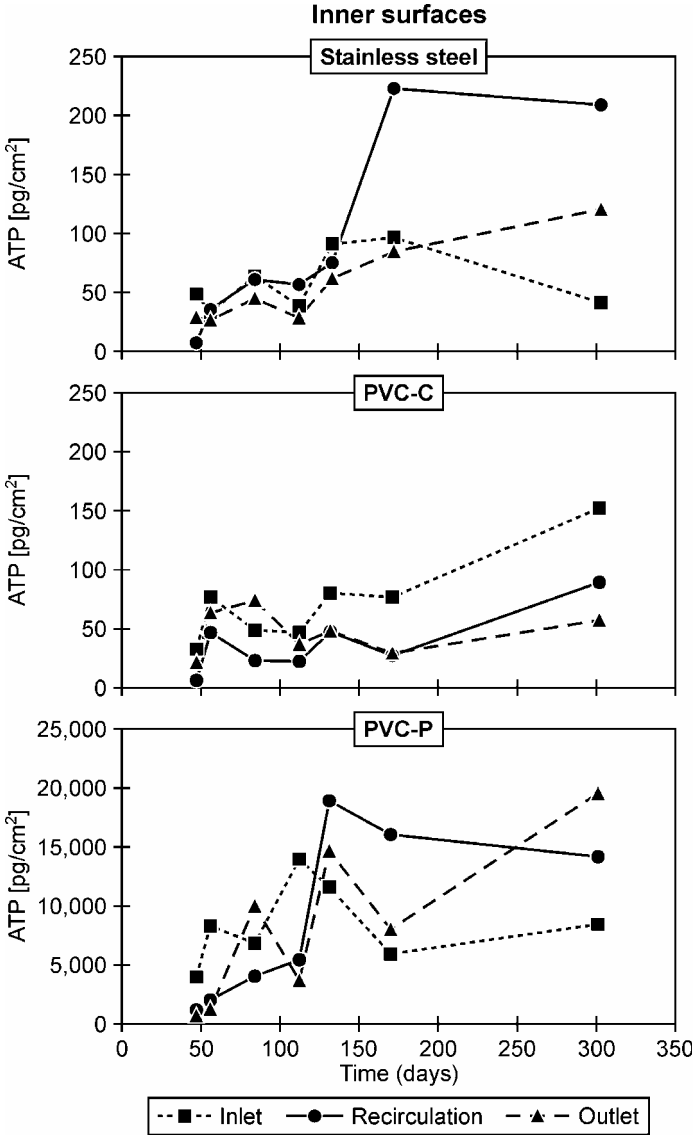

Figure 14: Biofilm formation expressed as ATP on surfaces of test pieces with only inner surfaces exposed to the water phase (single replicates) in the inlet line, in the recirculation section and in the outlet line of the flow model system. Investigations performed at 10 °C.

8 FROM BATCH AND MODEL TO 'THE REAL WORLD'

Optimising methods for investigating biofilm formation on polymers in laboratory and pilot scale still leaves the question, of how well results from such investigations correlate with reality in drinking water distribution systems. There is therefore a need for validation of results obtained in laboratory and pilot scale against biofilm densities measured in real distribution systems. Van der Kooij and Veenendaal (1993) measured biofilm densities on PVC pipe removed from three distribution systems and found values similar to values obtain on glass in the biofilm monitor. Also Hallam *et al.* (2001) found similar biofilm densities on nine pipes removed from a distribution system compared to what was observed in biofilm monitors with the same materials, but the results can be questioned, since the biofilm samples was collected from chlorinated systems. In order to translate investigation results to 'the real world', a more comprehensive knowledge regarding biofilm densities on different materials in distribution systems will be required.

In distribution systems distributing drinking water with high AOC values, the bioavailable migration from polymers has less impact on the microbial quality, since the growth promoting contribution from the water exceeds that of the polymers. Even in systems distributing biostable water, the migration of bioavailable compounds from polymers has less significance on the microbial quality of the water in the large diameter main pipes. Here the water flow is high and the S/V-ratio low, so the change in biomass in the water phase caused by polymers is small. In service pipes and house installations however, the pipe diameter is small, giving high S/V-ratios at a longer contact time due to the periodical tap patterns. Here the biomass concentration in the water phase may increase significantly as a result of the growth promoting properties of the polymers. For example in the investigation with PE at varying S/V-ratios (referred in paragraph 6.2.3) the biomass concentration in the water phase increased 840% at S/V-ratio 0.7 cm^{-1} (corresponding to an inner pipe diameter of 57 mm) with weekly water replacements compared to an increase of 260% at S/V-ratio 0.1 cm^{-1} (corresponding to an inner pipe diameter of 400 mm).

9 PROJECT CONCLUSIONS

Based on the experimental work described in the papers of the appendixes the following conclusions can be drawn:

ATP measurements proved to be a robust and sensitive tool for biomass measurements when investigating aftergrowth potential.

Biotic and abiotic testing both showed that the migration of bioavailable organic compounds is at an elevated level within the first weeks of use, followed by a lower but constant level. Investigation periods less than a month will thus likely overestimate the polymers aftergrowth potential during operation.

Biomass production during batch investigations of polymers is highly dependent of diversity and characteristics of the inoculum. Demands for inoculum composition are required to ensure uniform investigation results between investigations.

Investigations showed that the migration of bioavailable organic compounds from the surface of the polymers under sterile conditions is influenced by diffusion processes over the solid-liquid boundary layer. Thus diffusion-limitation with increasing S/V-ratios was observed under sterile conditions and the migration of bioavailable compounds increased with gentle shaking of the water phase. Due to the continuous consumption of bioavailable compounds, the driving force for the diffusion was kept high with an active biomass present, causing the migration of bioavailable compounds to be independent of S/V-ratios and gentle shaking of the water phase with the materials investigated. The presence of a biomass thus enhances the migration from the polymers, why it is important to perform investigation with an active biomass present in order not to underestimate the level of migration from polymers.

No water replacements versus replacements once a week or once every second week did not affect the migration of bioavailable compounds measured as biomass production by ATP and $HPC_{R2A,20C,28\ days}$ over a 16 weeks period, even if the biomass measured in the water phase in general accounted for up to 50% of the total biomass.

Temperature of 10ºC and 25ºC did not have a significant effect on the bioavailable migration measured as biomass production by ATP and $HPC_{R2A,20C,28\ days}$, but a tendency for higher biomass concentrations at 10ºC was observed.

Since the biofilm formation on polymers in flow model systems is supported by substrate from the material as well as from the water, special demands need to be fulfilled by flow model systems used for this purpose. A continues flow model system was developed optimised for investigation of bifilm formation on polymers under different combinations of flow velocity and contact time. Preliminary results with positive (PVC-P) and negative (stainless steel) controls and a material with low aftergrowth potential (PVC-C) showed good agreement between results obtained in batch set-ups and flow model systems within 16 weeks of incubation. A continuous increase in biofilm density on all three materials was observed over a test period of 43 weeks. Based on the three materials investigated no general conclusions on the effect of contact time and flow velocity could be made.

9.1 Future perspectives

In future investigations it will be important to clarify whatever the presence of biomass is capable of preventing diffusion limitation over the solid-liquid boundary layer regardless of the level of the polymers growth potential. If so, S/V-ratio will have no significance in batch investigations.

To get a complete understanding of which parameters have significant effect, it must be clarified to what degree the consumption of migration bioavailable compounds occurs within the biofilm. The extreme case would be that the mature biofilm is capable of consuming all bioavailable migration, regardless of the level of the polymers aftergrowth potential, making elevated biomass concentrations measured in the water phase solely a result of detachment from the biofilm. Demands to flow model systems will then be simplified, since no regard would have to be taken to material contact time and S/V-ratio.

Likewise need a number of materials with intermediate aftergrowth potentials to be investigated in optimised continues flow model systems, e.g. the one developed in this project to determine the significance of flow velocity combined with material contact time.

A structured collection of biofilm samples from real distribution systems, e.g. in connection with renewal and renovation, will give a greater understanding of polymers influence on microbial parameter during operation, and how to transfer test results from batch and flow model investigations to reality

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