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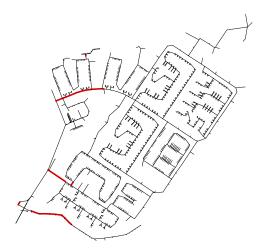
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BTO Managementsamenvatting

Een eerste stap naar een model voor nagroei in het distributienet

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Hoe groter de diameter van een leiding in het distributienet, hoe lager de concentratie biofilm die een klant mogelijkerwijze zal ontvangen. Dit blijkt uit een studie waarin de eerste stappen zijn gezet naar een model waarmee dichtheid en samenstelling van de biofilm en het effect daarvan op de waterkwaliteit in kaart kunnen worden gebracht. In Nederland wordt water van een uitstekende kwaliteit gedistribueerd zonder chloor. Het distributienet is als het ware een bioreactor; een omgeving voor nagroei. In deze studie zijn drie submodellen opgesteld voor het in beeld brengen van: (1) de instandhouding van de biofilm, (2) het loslaten van de biofilm, en (3) de omstandigheden waaronder dat loslaten optreedt. Dit gebeurde aan de hand van numerieke beschrijvingen en numerieke waardes van invoerparameters. Hieruit blijkt dat met name de leidingdiameter een bepalende rol speelt in het risico op biofilm in het water. Meer onderzoek is nodig naar het effect van hydraulische omstandigheden, temperatuur en concentratie voedingsstoffen in het leidingnet om de instandhouding en loslaten van de biofilm beter te begrijpen.



Aanwijzing van zelfreinigende leidingen (rood) in het distributienet waar het risico op loslaten van de biofilm klein is.

Belang: begrijpen van het distributienet als een bioreactor

In Nederland wordt water van een uitstekende kwaliteit gedistribueerd zonder chloor. Tijdens de distributie kan het distributienet worden opgevat als een bioreactor. Op de wand verkeert een biofilm in een 'steady state' en gaat mogelijk een uitwisseling aan met de waterfase. Voor een goede garantie van de waterkwaliteit is het is van belang om beter te begrijpen welke factoren de dichtheid en - in een later stadium - de samenstelling van de biofilm beïnvloeden, evenals het effect daarvan op de waterkwaliteit bij de klant. Een model dat de

hoeveelheid biofilm in het distributienet beschrijft is daarvoor noodzakelijk.

Aanpak: conceptueel en numeriek model van instandhouding en loslaten biofilm

Als eerste stap in het project is een conceptueel model opgesteld, bestaande uit drie submodellen: (1) de instandhouding (maintenance) van de biofilm (steady state), (2) het loslaten van de biofilm (of netto uitwisseling tussen biofilm en waterfase), en (3) de omstandigheden waaronder de biofilm loslaat. In een vervolgstap is gezocht naar literatuur waarmee de conceptuele modellen ook naar numerieke beschrijvingen zijn te vertalen.

In de laatste stap binnen dit project zijn numerieke waardes van parameters in de modellen achterhaald. Daarna is een kleine verkenning gedaan om het numerieke model ook toe te passen op een leidingnetmodel van een wijk in Nederland.

Resultaten: meer inzicht, maar beperkte beschikbaarheid van modellen

Voor de instandhouding van een biofilm in het leidingnet (submodel 1) geldt de concentratie voedingsstoffen in het water (met als drijvende kracht diffusie) als bepalende factor. Bij een bepaalde concentratie voedingsstoffen, die ook nog zeer beperkt afneemt over de lengte van het leidingnet, is de biofilmdichtheid overal gelijk. Stroomsnelheid en leidingdiameter spelen in dit geval geen rol. Over het effect van de temperatuur, en de schuifspanning op de sterkte van de biofilmstructuur is onvoldoende bekend. Theoretisch gezien zou onder een constante voedingsstoffenconcentratie bij hogere temperaturen een groter deel van de biofilm kunnen loslaten omdat de diffusie minder snel toeneemt dan de stofwisseling van de biofilm. Voor het loslaten van de biofilm (submodel 2) is gebruikgemaakt van PODDS, een model ontwikkeld aan de universiteit van Sheffield waarin tevens rekening wordt gehouden met sediment. De theorie achter dit model suggereert dat bij hogere schuifspanningen tijdens reguliere omstandigheden in het net, een kleine verstoring al kan leiden tot het vrijkomen van een groot deel van de relatief 'losse' biofilm. Onduidelijk is of de veronderstelde uitwerking van schuifspanning ook geldt voor biofilm zonder of met heel weinig sediment. Ook voorspelt dit model dat hydraulische verstoringen in zelfreinigende netten nooit de oorzaak kunnen zijn van het loslaten van de biofilm. Deze aspecten moeten getest worden in een echt

Voor de omstandigheden waaronder de biofilm loslaat (submodel 3) is het nodig om naar de samenhang met het voorgaande submodel 2 te kijken. Het gaat in dit geval om hydraulische verstoringen als gevolg van leidingbreuken, openen van brandkranen of sterke toename van (huishoudelijk) waterverbruik. Met een stochastisch model kunnen de kans op deze verstoringen en bijbehorende locaties worden verwerkt. Wegens bedenkingen bij submodel 2 en onbekendheid van waterbedrijven met numerieke waardes van de invoerparameters voor submodel 3 (frequentie optreden van verstoringen in samenhang met bijvoorbeeld diameter of materiaalsoort van het leidingnet), is dit submodel nog niet verder uitgewerkt.

Op grond van bovenstaande bevindingen lijkt het risico – de hoeveelheid biofilm als concentratie die een klant zou kunnen ontvangen – met name te worden bepaald door de diameter: een grotere diameter leidt tot een lager risico.

Implementatie: toewerken naar een tool voor modelleren fysische én biologische vervuiling

Voor submodel 1 is meer inzicht nodig in parameters die de maintenance van de biofilm beïnvloeden. Komende jaren verwachten we resultaten van labonderzoek bij KWR en de Universiteit van Sheffield. Daarna is verdere ontwikkeling van het model mogelijk. Ook wordt de komende jaren in het BTO een vervuilingsvoorspellingstool ontwikkeld dat zich in eerste instantie alleen richt op bruinwaterrisico en dus op deeltjes. In een volgende stap kan dit model worden uitgebreid met het model voor de biofilm. Daarmee is het relatief eenvoudig om het nagroeimodel toepasbaar te maken op een volledig leidingnet, wat de gebruiker een tool in handen geeft om zowel de fysische als de biologische vervuiling te modelleren.

Rapport

Dit onderzoek is beschreven in rapport *Modelling* growth in the distribution network (BTO-2017.077).







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1 Modelling bacterial biomass in a non-chlorinated drinking water distribution system

In the Netherlands, the distributed drinking water does not rely on residual disinfectants. To control and limit growth of microorganisms, the drinking water is produced with low concentrations of degradable compounds (e.g. assimilable organic carbon, AOC). Still, dynamics of increase and decrease of microorganisms in the water (biomass) during residence in the drinking water distribution system (DWDS) has been observed (van der Wielen et al. 2016; van der Wielen and van der Kooij 2013; van der Wielen and van der Kooij 2010). Microorganisms can multiply and accumulate in the planktonic phase but also on the interface between the water and surfaces (particulate material and the pipe wall) in the DWDS, leading to the development of biofilms. More than 95% of the biomass in the drinking water distribution system is associated with biofilm (Liu et al. 2014). In addition, it has been observed that the total microbial biomass and the dominant bacterial composition remains stable during distribution to the consumers (Roeselers et al. 2015; van der Wielen et al. 2016). This stability is probably due to the fact that under normal operational circumstances in the DWDS the biofilm is in equilibrium with its environment and there is little net exchange of biomass between water and biofilm. However, in case of a disturbance of the equilibrium there is a risk of biofilm detachment which may result in discolouration and/or detachment of biofilm related opportunistic pathogens such as Legionella pneumophila or Pseudomonas aeruginosa.

Drinking water companies want to avoid these undesirable effects. In a risk based approach information is required on (i) the probability of biomass detachment and (ii) the effect of biomass detachment. This requires determining when and how often disturbances can lead to biomass detachment and understanding where biofilm is located in the DWDS, what the biofilm density is and the amount of biofilm that can be detached, depending on the type and magnitude of the disturbance. The results from such a risk-based approach can then be used to develop and evaluate mitigation strategies. Research has been done on biofilm formation and detachment, mainly under laboratory conditions in static batch tests, but also under dynamic conditions using test rigs of water mains. The biofilm most probably interacts with particulate matter ('sediments') in het DWDS. This interaction complicates matters, and will not be taken into account explicitly.

Several researchers have tried to develop a model of biofilm progress in the DWDS. These models are either statistical models (e.g. Pinto et al. 2012), based on regression analyses, or deterministic models, based on a theoretical approach (e.g. RIGA (Rubulis et al. 2007), ZHANG (Zhang et al. 2004), SANCHO (Servais et al. 1995), and PICCOBIO (Dukan et al. 1996)). Both types of models have pros and cons, resulting from the different approaches that are used. Where statistical models are based on observations, deterministic models are based on understanding the physics, chemistry and biology, which are challenged by the complexity of the biological processes in a DWDS (van Lieverloo et al. 2012). Although existing models serve as a valuable starting point, there are some drawbacks. Most available models are very complex and often contain (too) many interdependent parameters, making it difficult to validate them (Rubulis et al. 2007). Even if good predictions are obtained it remains

uncertain whether good predictions will be obtained under different conditions, making these models less suited for sensitivity analyses or water management. Also, all these existing models are applied for drinking water that is distributed with a residual disinfectant, which is not compatible with the specific biological conditions in the network without residual disinfectants as is the case in the Netherlands. Other drawbacks of these existing models are: (i) calculating times can be substantial and not all of these models are translated into a numerical water quality model that can be linked to a hydraulic calculation and (ii) that they only consider biofilm growth, but not detachment. A model that does take into account both growth and detachment is VCDM (Variable Condition Discoloration Model, from the University of Sheffield). However, it models growth and detachment of cohesive layers which are perceived to be existing of a combination of particulate matter and biofilm. Upon detachment of the cohesive layer an increase in turbidity is measurable. VCDM is validated with turbidity measurements, and no specific biomass measurements (Furnass et al. 2014; Furnass 2015). Also, VCDM is a data driven approach, not based on (biological) growth models. Furthermore, VCDM has so far only be applied on trunk mains, not on the distribution part of the DWDS. None of the models, as such, are fully applicable for the risk based approach, because they do not consider all the relevant aspects of biofilm development, detachment and hydraulic conditions.

The ultimate goal is to develop a risk based approach for biofilm detachment in a non-chlorinated DWDS. Therefore, we need to describe for specific DWDS circumstances (pipe material, pipe diameter, hydraulic circumstances, incoming water quality, etc.) the magnitude of biofilm present, the part of the biofilm that can be detached, and the probability of disturbances that will lead to detachment. Such a model will be a first step that will help to identify which processes are the most important and, as a result, need to be developed in more detail. This sensitivity analysis can be done by applying the model on a real DWDS. In addition, by applying the model to a real DWDS, the assumptions that we use in the model can also be validated.

This report describes the first steps in working towards this ultimate goal. It describes the conceptual (§ 2.1) and numerical model (§ 2.5) that we developed to describe the risk of biofilm detachment, based on models for biofilm maintenance (§ 2.2), and effect and probability of hydraulic circumstance that would detach biofilm (§ 2.3 and 2.4 respectively). These models are based on the literature, and still need validation. Chapter 3 discusses some of the required and planned validation measurements.

2 Methods and materials

2.1 Conceptual model

The aim is to develop a risk based approach for the biofilm detachment in a DWDS, with its variability in flow conditions, diameters, local water quality, pipe materials etc. The starting point for the conceptual model is that under normal operational circumstances in the DWDS there will always be a biofilm, and that this biofilm is in equilibrium with its environment, i.e. there is little net exchange of biomass between the biofilm and the drinking water (which will be called steady state biofilm in this paragraph). The steady state biofilm in equilibrium may be affected by local circumstances in the DWDS, such as pipe material and pipe diameter, flow velocities or shear stress on the pipe wall, the temperature of the water, and the water quality (e.g. AOC). It is assumed that under normal daily operating conditions, the microbial biomass in the network is stable and shows low variation, only on a larger time scale the biomass will show slow adaptations (seasonal fluctuation).

In case of a disturbance of the steady state there is a risk of biomass detachment. In our model we shall only consider sudden disturbances, i.e. changes over a short time period. This means that at a certain location in a DWDS pipe material and pipe diameter are considered to be constant and do not disturb the steady state. Sudden, out of the ordinary, changes in flow can disturb the steady state. Potentially also changes in temperature and incoming water quality can disturb the steady state. As temperature in the DWDS is mostly affected by the temperature of the soil surrounding the pipe (Blokker and Pieterse-Quirijns 2013), and at 1 meter depth the soil temperature changes gradually with maximum 1 °C per day (Agudelo-Vera et al. 2015), a sudden change in temperature is not to be expected, and will be discarded. Also, a sudden change in (local) water quality, e.g. due to contamination of the DWDS will for now not be considered, as the likelihood of this is still very uncertain (Besner et al. 2011; Blokker et al. 2014; van Lieverloo et al. 2007). This means that we shall mainly focus on hydraulic disturbances, such as changes in flows due to bursts, valve closures for mains repairs, large increase in demand, and flushing events.

An alternative hypothesis is that enhanced exchange between biofilm and water will take place during long contact times, i.e. during the time that there is stagnancy of the water. Again, we would consider this effect only in case this no flow situation takes place under abnormal (disturbance) circumstances. Under normal operational conditions we shall assume a steady state even in no (or low) flow circumstances, and when hydraulics change in such a way that long contact times occur this can serve as a hydraulic disturbance. A sudden change to long contact times will only occur if valves are closed or opened in the DWDS. In the case of unusual long contact times this water will not supply any customers, and therefor the risk of biomass reaching the customer will be zero. Therefor the effect of biofilm detachment due to long contact times is neglected for now.

We consider the measurable biomass concentration as the main model parameter. Total active bacterial biomass in biofilm and water is measured as adenosine triphosphate (ATP) (in g), which has been proven to be an apt measure for the amount of active biomass in drinking water and biofilms in non-chlorinated drinking water (van der Kooij 1992; Van der Kooij et al. 1995b). In the literature biomass is typical expressed in mg C; there is a linear factor between the two, and both units are used in this report.

2.2 Biofilm formation and maintenance in the DWDS

Biofilm formation on a distribution pipe will start immediately after water is flowing through the pipe. The first step in this biofilm development is the formation of a conditioning film, consisting of macromolecules (such as polysaccharides, lipids, proteins and humic substances) and ions that adhere to the surface of the pipe wall due to either physical or chemical processes (Liu et al. 2016; Manuel 2007). The next step is the adhesion of microorganisms to the conditioning film, mainly due to electrostatic interactions and Van-der Waals forces (Manuel 2007). The adhered bacteria subsequently form microcolonies, with the production of EPS and quorum-sensing molecules (Liu et al. 2016). The microbial growth and EPS production results in an increasing biofilm concentration, which is based on the (amount of) substrate that is available (van der Kooij and Veenendaal 2014) after which the biofilm concentration remains stable (Van der Kooij et al. 2003).

During the biofilm formation process in the drinking water environment, different growth phases can be distinguished. The first few days after surfaces are exposed to nonchlorinated drinking water the biofilm formation is exponential, followed by a linear biofilm increase for approximately an additional 90 days after which the biofilm concentration remains stable in time (Van der Kooij et al. 1995a), which we have defined as the maintenance phase. Rittmann and McCarty (1980) have shown that this is steady state is related to diffusion. The majority of the pipes in a DWDS have been in operation for more than 100 days and, consequently, the biofilm concentration on these pipes have a stable concentration over time. In the maintenance phase the biofilm concentration remains stable while four processes occur during this phase: (i) micro-organisms in the biofilm decay and their biomass is replaced by microbial growth, (ii) protozoans graze on the biofilm and bacterial loss by grazing is complemented by bacterial growth, (iii) biofilm is released in the water and complemented by microbial growth, (iv) micro-organisms gain just enough energy from the low nutrient concentrations in drinking water environment to run their cell maintenance processes (i.e. cell respiration) (McGrew and Mallette 1962). In order to model the maintenance phase of the biofilm, it is important to identify the dominant process. Boe-Hansen et al. (2003) demonstrated that bacterial decay in the biofilm (the 1st process above) is a negligible or minor process. Their research was done on biofilms that were formed for more than 200 days on distribution pipes in a model distribution systems which was supplied with drinking water without a disinfectant residual; the results showed that virtually all cells in the biofilm were viable. Several studies have indicated that protozoans are among the common microflora in the drinking water distribution system (van Lieverloo et al. 2002 and referencs therein). However, these protozoan cells can gain just enough energy to maintain their biomass, since the biofilm concentration remains stable in the maintenance phase. This means the 2nd process is of limited importance. Research on the bacterial communities using next generation sequencing of non-chlorinated drinking water and biofilms from pipe surfaces demonstrated that the bacteria present in biofilms differ from the ones in the drinking water (Liu et al. 2014; Roeselers et al. 2015). This indicates that biofilm release (the 3rd process above) does not seems to be a dominant process in the DWDS under normal operating conditions. The research was done on relatively young biofilms, and it may be that the first two processes may be more important in older biofilms.

Overall, it can be concluded from the results of these studies that biofilm decay and release is limited, and it is, therefore, suggested that cell maintenance processes (4th process above) is the most dominant in maintaining the biofilm during the maintenance phase. Some studies have investigated whether microbial cell respiration or microbial cell growth dominates in biofilms. Boe-Hansen et al. (2003) showed that the consumption rate of AOC of a drinking water biofilm was 100 times higher than the protein biomass production rate, which demonstrates that cell respiration is large compared to cell growth. In addition, [14C]-

labelled benzoic acid injection into the same model distribution system further confirmed this observation. Furthermore, another study demonstrated that more than approximately 90% of the biomass in a stable biofilm in a microbial fuel cell is respiring (Jayasinghe et al. 2014). Therefore, cell maintenance is assumed to be the determining process during the maintenance phase of the biofilm.

Pirt (1982) has shown that the required substrate mass for biofilm maintenance can be described with the help of a maintenance coefficient ($\mu_{\rm m}$). This maintenance coefficient describes the amount of substrate (mg_{c,s}) needed per day to maintain a certain biomass concentration (mg_{c,s}) at a certain level and is given in mg_{c,s}.mg⁻¹_{c,s}.day⁻¹. It can be deduced from the maintenance coefficient that the substrate uptake ($\Delta C_{\rm s}$, in mg_{c,s}.mr⁻³) can be expressed as:

$$\Delta C_S = \frac{\mu_m \cdot M_B \cdot \Delta t}{V} \tag{1}$$

In which $M_{\rm B}$ is the bacterial biomass (mg_{C,B}), Δt is the contact time (day) and V is volume (m³). For a certain pipe with length L (m) and diameter D (m), $M_{\rm B}$ and $\rho_{\rm B}$, the bacterial biomass per surface area (mg_{C,B}·m²) or biofilm density, are related through the surface area of the pipe wall. The time Δt is given by:

$$\Delta t = \frac{\Delta L}{t^2} \tag{2}$$

where v the flow rate (m.day⁻¹) and ΔL the pipe length (m) over which the biofilm is considered. Substituting these in equation (1) gives:

$$\Delta C_S = \frac{\mu_m \cdot \rho_B \cdot A \cdot \frac{\Delta L}{v}}{V} = \frac{\mu_m \cdot \rho_B \cdot \pi DL \cdot \frac{\Delta L}{v}}{\pi (D/2)^2 L} = \frac{4\mu_m \cdot \rho_B \cdot \Delta L}{D \cdot v}$$
(3)

To maintain a certain biofilm density over the pipe length, we can determine the required substrate uptake per day or per meter as:

$$\frac{\Delta C_S}{\Delta t} = \frac{4\mu_m \cdot \rho_B}{D} \tag{4}$$

$$\frac{\Delta C_S}{\Delta L} = \frac{4\mu_m \cdot \rho_B}{D \cdot \nu} \tag{5}$$

The biofilm density $\rho_{\rm B}$ was determined on 39 PVC-U pipes that were dug up from the DWDS of 15 supply zones that retrieved drinking water from different treatment plants. A defined area of the pipe was swabbed and subsequently the adenosine triphosphate (ATP) density (a measure for biomass) was determined and values were expressed as ng ATP m⁻². The ATP density varied between 40 and 42,650 ng ATP m⁻², with an average density of 5852 ng ATP m⁻². Karl (1980) has observed an average C to ATP ratio of 250 mg C/mg ATP for bacteria and using this ratio a bacterial biomass density ($\rho_{\rm B}$) of 0.010 to 10.7 mg_{C,B}·m⁻² (average 1.46 mg_{C,B}·m⁻²) has been calculated for the biofilm on the pipe wall in a DWDS where drinking water without a disinfectant residual is distributed.

The maintenance coefficient depends on the temperature and energy source used by the microorganisms. Tijhuis et al. (1993) derived a relation between biomass specific maintenance rate of Gibbs energy dissipation (m_E in KJ mol C^{-1} h⁻¹) and temperature (T in K) for aerobic growth:

$$m_E = 5.7e^{\left\{\frac{-6.94 \times 10^4}{R} \times \left(\frac{1}{T} - \frac{1}{298}\right)\right\}}$$
 (6)

With R the gas constant (8.314 kJ/K). The $m_{\rm E}$ is similar to the maintenance coefficient $\mu_{\rm m}$ except that the $\mu_{\rm m}$ is expressed in mg C (S) per mg C (B) per day. The following equation converts $m_{\rm E}$ into $\mu_{\rm m}$:

$$\mu_m = \frac{m_E}{12} \times 24 \times \left(\frac{\Delta G_{av}^{01} \times \gamma_D}{12}\right)^{-1} \tag{7}$$

where $\Delta G_{av}^{0.1}$ is the average available Gibbs energy in the electron donor/acceptor couple used for growth (kJ mol electrons⁻¹) and γ_D the degree of reduction of the electron donor used for growth (dimensionless). The factor 12 is used in order to convert mmol to $mg_{c,B}$, and the factor 24 to convert hours to days. Substituting Eq. (6) in equation (7) gives:

$$\mu_m = \frac{5.7e^{\left\{\frac{-6.94 \times 10^4}{R} \times \left(\frac{1}{T} - \frac{1}{298}\right)\right\}}}{\Delta G_{av}^{01} \times \gamma_D} \times 24$$
 (8)

The μ was calculated for different growth substrates at temperatures between 10 and 25°C (Table 1). The results show that α varies between 0.026 (carbohydrates at 10°C) and 1.04 mg_{CS}.mg⁻¹_{CR}.day⁻¹ (oxalate at 25°C).

TABLE 1. $\mathrm{m_{_E}}$ AND $\mu_{_{\!\! m}}$ VALUES FOR VARIOUS SUBSTRATES AND TEMPERATURES.

Substrate	ΔG_{av}^{01} (kJ mol electrons ⁻¹)	γ _D (-)	T (°C)	<i>m</i> _E (KJ mol C ⁻¹ h ⁻¹)	μ <u></u> (mg mg ¹ day ¹)	Reference
Oxalate	131	1	10	1.29	0.237	(Tijhuis et al. 1993)
			15	2.16	0.395	<u>.</u>
			20	3.53	0.647	
			25	5.70	1.04	
Glucose	118.6	4	10	1.29	0.065	(Tijhuis et al. 1993)
			15	2.16	0.109	
			20	3.53	0.179	
			25	5.70	0.29	
Succinate	107.3	3.5	10	1.29	0.083	(Tijhuis et al. 1993)
			15	2.16	0.138	
			20	3.53	0.226	
			25	5.70	0.36	
Malate	112.3	3	10	1.29	0.092	(Tijhuis et al. 1993)
			15	2.16	0.154	
			20	3.53	0.252	
			25	5.70	0.41	
Mannitol	117.7	4.33	10	1.29	0.061	(Tijhuis et al. 1993)
			15	2.16	0.101	
			20	3.53	0.166	
			25	5.70	0.27	
Acetate	105.5	4	10	1.29	0.073	(Heijnen and Van Dijken 1992)
			15	2.16	0.123	
			20	3.53	0.201	
			25	5.70	0.324	
Proteins	110.4	10	10	1.29	0.028	(Comeau 2008),
			15	2.16	0.047	$\square_{_{D}}$ assumed
			20	3.53	0.077	-
			25	5.70	0.124	-
Carbo-	120	10	10	1.29	0.026	(Comeau 2008)
hydrates			15	2.16	0.043	_ 🗆 _ assumed
			20	3.53	0.071	• -
			25	5.70	0.114	•

From Eqs. (4) and (5) $\rho_{\rm g}$ can be determined when the substrate uptake is known. However, in typical Dutch DWDS with an AOC of 1.0 to 10 $\mu{\rm g}$ C/l in treated water entering the DWDS, AOC levels after a certain pipe length of several km or water residence time of several days do not show a measurable decrease (van der Kooij 1992). This means that $\Delta C_{\rm g}$ is not known but is low (< 1.0 $\mu{\rm g}$ C/l). Rittmann and McCarty (1980) describe how the biofilm density is maintained by a substrate flux (J, mg_{C,S}·m⁻²·day⁻¹), which is solely driven by molecular diffusion of the substrate into the biofilm, and thus is affected by the substrate concentration in the water. For Eqs. (4) and (5) this means that $\Delta C_{\rm g}$ is a function of $C_{\rm g}$ which can be measured at the water treatment works.

They state that

- For low values of C_s , there is no possibility to maintain a steady state biofilm (J = 0, $\rho_B = 0$).
- For high values of C_s there is a so called deep biofilm, and it should be relatively easy to determine its density.
- For medium high values of C_s there is a so called shallow biofilm, and it it is more difficult to determine its density. Shallow biofilms need a relatively large substrate flux from the available C_s for maintenance, compared to deep biofilms; i.e. the proportion of the available substrate that is used for maintenance ($\Delta C_s / C_s$) is larger for shallow biofilms than for deep biofilms.

In a typical DWDS only shallow biofilms (1 to 10 cells deep) are found. For a shallow biofilm (and thus for $C_{\rm s,min} < C_{\rm s} < C_{\rm s,deep}$) the following holds

$$J = J_{deep} \left(\frac{C_S - C_{S,min}}{C_{S,deep} - C_{S,min}} \right) = \phi \left(C_S - C_{S,min} \right)$$
(9)

$$\phi = \frac{J_{deep}}{C_{S,deep} - C_{S,min}} \tag{10}$$

with J the substrate flux (mg_{c,s}.m⁻².day⁻¹) for a shallow biofilm and J_{deep} for a deep biofilm, C_s the substrate concentration (mg_{c,s}.m⁻³), $C_{s,min}$ the minimum substrate concentration to maintain a biofilm, $C_{s,deep}$ the substrate concentration to maintain a deep biofilm, and ϕ a conversion factor (m.day⁻¹). This conversion factor is not given by Rittmann and McCarty (1980), but it can be derived from their text that there should be such a factor. This conversion factor has the unit of velocity, so is probably related to the diffusion coefficient (in m².s⁻¹ or m².day⁻¹).

The substrate uptake per day is determined by:

$$\frac{\Delta C_S}{\Delta t} = \frac{J \cdot A}{V} = J \frac{\pi D L}{\pi (\frac{D}{2})^2 L} = \frac{4J}{D} = \frac{4\phi (C_S - C_{S,min})}{D}$$
(11)

Comparing this to Eq. (4) we find that the biofilm density that can be maintained is related to the available substrate, the maintenance rate and the factor ϕ :

$$\rho_B = \frac{\phi(C_S - C_{S,min})}{\mu_m} \tag{12}$$

And the substrate concentration over time can be determined from

$$C_{S,t+\Delta t} = C_S - \Delta C_S = C_{S,t} - \frac{4\phi(C_{S,t} - C_{S,min})}{D} = \frac{4\phi}{D}C_{S,min} + C_{S,t}\left(1 - \frac{4\phi}{D}\right)$$
(13)

$$C_S(t) = C_{S,min} + (C_S(0) - C_{S,min})e^{-\frac{4\phi}{D}t}$$
(14)

In the Netherlands we do find a steady state biofilm (40 ng ATPm²) for AOC levels below 1 μ g C/l; suggesting that $C_{s,min}$ may be approximated with 0. A numerical example can shed some light on the meaning of these equations:

- Using the lower values for μ_m (Table 1, minimum value at 15 °C: 0.04 mg_{c,s}.mg⁻¹.day⁻¹) and $\rho_{\rm g}$ (0.01 mg_{c,g}.m⁻²), $C_{\rm s}=5$ mg_{c,s}.m⁻³ (5 μ g.l⁻¹ AOC) and $C_{\rm s,min}=0.1$ mg_{c,s}.m⁻³ (0.1 μ g.l⁻¹ AOC), ϕ can be estimated as 0.09 mm.day⁻¹. Using a pipe diameter of 100 mm and a residence time of two days in the DWDS, the result is a ca. 1% decrease in AOC, which would not be measurable (blue line in Figure 1).
- Using more realistic values for μ_m (Table 1, mean value at 15 °C: 0.13 mg_{C,S}.mg⁻¹.day⁻¹) and ρ_B (1.46 mg_{C,B}.m⁻²), $C_S = 5$ mg_{C,S}.m⁻³ (5 μ g.l⁻¹ AOC) and $C_{S,min} = 0.1$ mg_{C,S}.m⁻³ (0.1 μ g.l⁻¹ AOC), ϕ can be estimated as 41.7 mm.day⁻¹. Using a pipe diameter of 100 mm and a residence time of two days in the DWDS, the result is a ca. 95% decrease in AOC, which would be measurable (red line in Figure 1).
- Using high values for $\mu_{\rm m}$ (Table 1, max value at 15 °C: 0.40 mg_{c,s}.mg¹.day¹) and $\rho_{\rm B}$ (10.7 mg_{c,s}.m²), $C_{\rm s}=5$ mg_{c,s}.m³ (5 μ g.l¹ AOC) and $C_{\rm s,min}=0.1$ mg_{c,s}.m³ (0.1 μ g.l¹ AOC), ϕ can be estimated as 862 mm.day¹, which is comparable to the values from (Rittmann and McCarty 1980). Using a pipe diameter of 100 mm and a residence time of two days in the DWDS, the result is a ca. 98% decrease in AOC, which would be measurable (green line in Figure 1).

Typically in the Netherlands no decrease in substrate is measured over the length of a DWDS. This corresponds to the blue line in Figure 1. This means that Eq. (14) can be simplified to a constant, $C_s(t) = C_s(0)$. Thus, Eq. (12) does not depend on time, and ρ_B does not vary with the travel time in the DWDS.

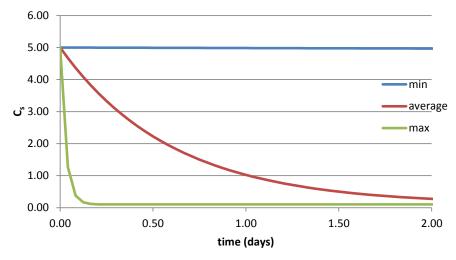


FIGURE 1. EXAMPLE OF SUBSTRATE DECAY OVER TIME, EQ. (14)

We have not been able to determine the numerical values of ϕ from the literature. For now we have to conclude that the value of ϕ is not known for biofilms in a DWDS in the Netherlands. It should be small, as no decrease in substrate is typically measured over the length of a DWDS. An attempt of determining some correlations between the parameters (e.g. diffusion constant, pipe diameter, and substrate concentration) was restricted due to the available literature, see appendix I.

The literature suggests that ϕ (m.day⁻¹) only depends on the substrate concentration and diffusivity, but there is no known dependence on the flow shear stress or pipe diameter. So starting from Eq. (12), and given the fact that C_{ς} does not measurably decrease over the

DWDS (the blue line in Figure 1), the biofilm density does not vary over the DWDS with changes in velocity or diameter. This means that once a steady state biofilm is reached, the substrate concentration remains sufficient to maintain the steady state biofilm and the contact time is always long enough for the biofilm density to be maintained as well. As a result, the parameters flow velocity and contact time do not explicitly appear in the equations needed in the model.

When all of the biofilm is detached and comes into the drinking water, the biomass concentration C_{R} (mg.m⁻³) in the water will be equal to:

$$C_B = \frac{M_B}{V} = \frac{\rho_B \cdot \pi DL}{\pi \left(\frac{D}{2}\right)^2 L} = \frac{4\rho_B}{D}$$
 (15)

This means that the larger the pipe diameter, the less effect a detached biofilm has expressed as the biomass concentration in the water. This would suggest that the "risk of biofilm detachment" is inversely proportional to the pipe diameter only. Whether all of the biofilm will come off, or just a part is subject of Sections 2.3 and 2.4.

TABLE 2. SYMBOLS USED IN EQAUTIONS, MASSES IN mg C.

symbol	unity	description	typical value	reference
D	[m]	Pipe diameter		
L	[m]	Length of pipe		
Α	[m²]	Surface of pipe wall		$A = \pi DL$
v	[m³]	Volume of pipe		V = 1/4 π
	[1]	Florence le cite de crise		D ² L
v	[m.s ⁻¹]	Flow velocity in pipe		
t	[day]	Time		
М	[mg]	Mass (subscript S for substrate, B for biomass)		
С	[mg.m ⁻³]	Concentration (subscript S for substrate, B for biomass)		C = M/V
$\Delta C_{\rm S}$	[mg _{c,s} .m ⁻³]	Substrate uptake		
ρ	[mg.m ⁻²]	Surface density (subscript B for biomass)	0.010 - 10.7	$\rho = M/A$ This study
$\mu_{_{\mathrm{m}}}$	[mg _{c,s} . mg ⁻¹ _c,g.day ⁻¹]	Maintenance coefficient	0.026 - 1.04	Table 1
m _E	[KJ mol C ⁻¹	Gibbs energy dissipation	1.3 - 5.7	Table 1
Т	[K]	Temperature	283 - 298	
R	[kJ.K ⁻¹]	Gas constant	8.314	
ΔG_{av}^{01}	[kJ mol ·1]	the average available Gibbs energy in the electron donor/acceptor couple used for growth	100 - 130	Table 1
7 _D	[-]	the degree of reduction of the electron donor used for growth	1 - 10	Table 1
S	[mg _{c,s} .m ⁻³]	rate limiting substrate concentration (minimum, deep, shallow)		
J	[mg _{c,s} -m ⁻² . day ⁻¹]	Substrate flux		
φ	[m.day ⁻¹]	Conversion factor to describe substrate flux as a function of substrate concentration		

2.3 Biofilm detachment: effect of disturbances

It is assumed that the biofilm can be eroded by applying a high enough shear stress. The relation between biofilm strength, due to operation conditions, and shear stresses during a hydraulic disturbance has been studied in the lab and the field, leading to the PODDS modelling approach (Boxall and Saul 2005; Boxall et al. 2003b; Husband and Boxall 2011; Husband et al. 2008; Husband and Boxall 2009; Husband and Boxall 2016). PODDS (prediction and control of discolouration in distribution systems) was first aimed at discolouration. It was shown that the discolouration material also contains biofilm, or that biofilm contains discolouration material (because the material sticks to the biofilm). Several studies have shown that there may be a (linear) correlation between turbidity in the flushing water (as a measure of discolouration) and various biofilm indicators (total cell count, ATP, etc.) (Besner et al. 2012; Boxall et al. 2003a; Schaap and Blokker 2012; Vreeburg and Beverloo 2011; Vreeburg et al. 2008). This suggests that the PODDS model may also be used as a concept for modelling biofilm detachment.

In the PODDS concept there is a build-up of discolouration material depending on the maximum applied shear stress (referred to as the conditioning shear stress); when a hydraulic disturbance with a shear stress above this value is applied, discolouration material (plus biofilm) can be suspended, leading to a discolouration event. This event is measured as turbidity and expressed in NTU. Furthermore, there is a shear stress above which no extra discolouration material is being suspended. There is a first order relation between turbidity and shear stress as in Figure 2. However, it is commonly accepted that no matter how high the shear stress, it will not be possible to remove all of the biofilm. This "maximum shear stress" should thus be considered to lead to a maximum exchange between biofilm and water, not as the removal of all biofilm.

In a DWDS the PODDS "conditioning shear stress" is the maximum shear stress that is experienced during normal operation, e.g. during the maximum hour in a week or month. In the concept of self-cleaning networks it is assumed that a regularly occurring high flow velocity ensures that during hydraulic disturbances (even higher flow velocities) no discolouration material is being suspended (Blokker et al. 2007b; Vreeburg et al. 2009). We assume the PODDS "maximum shear stress" to be equal to the self-cleaning shear stress. This means that it is possible to have a conditioning shear stress without build-up of discolouration material. A maximum velocity of 0.2 to 0.25 m/s during 50% of the days leads to self-cleaning pipes in a DWDS of 80 to 250 mm diameter AC pipes (Blokker et al. 2010). This corresponds to a shear stress of ca. 0.13 N/m². Given the concept of Figure 2, it would also be possible to determine the self-cleaning shear stress by determining the minimum required flushing velocity. Friedman et al. (2003) found it very difficult to establish this minimum flushing velocity and did not come to a single value. Husband and Boxall (2009) found that in chlorinated systems in the UK 0.6 m/s is enough to flush plastic pipes with a diameter of 50 to 200 mm, which corresponds to a shear stress of ca. 1 N/m². The slope of the line between the conditioning and self-cleaning shear stress is equal to "k", and it's value may depend on pipe diameter. Once the value of k is known the absolute values of the turbidity potential can be assessed. In our model approach this potential is described by the model of Section 2.2, and the PODDS concept is only used to determine the relative effect of a hydraulic disturbance. For the relative effect the exact value of k is not important.

The turbidity potential is determined by:

$$T_{conditioning} = k \times (\tau_{conditioning} - \tau_{self_cleaning})$$
 (16)

The turbidity effect of a hydraulic disturbance can be calculated as follows:

$$\Delta T = k \times \left(\tau_{conditioning} - min(\tau_{disturbance}, \tau_{self_cleaning})\right)$$
 (17)

This turbidity effect can be expressed as a fraction of the potential:

$$\frac{\Delta T}{T_{conditioning}} = \begin{cases} \frac{\left(\tau_{disturbance} - \tau_{conditioning}\right)}{\left(\tau_{self_cleaning} - \tau_{conditioning}\right)}, & if \ \tau_c \leq \tau_d \leq \tau_{sc} \\ 1, & if \ \tau_c \leq \tau_{sc} \leq \tau_d \\ 0, & if \ \tau_{sc} \leq \tau_c \end{cases}$$

$$(18)$$

When the conditioning shear stress is above the self-cleaning shear stress, the fraction of the potential suspension of discolouration material or biofilm removal is equal to 0. If the disturbance shear stress is above the self-cleaning shear stress, the fraction is equal to 1. Otherwise, the fraction is between 0 and 1. This concept also means that in order to have a certain fraction the extra shear stress required ($\tau_{\text{disturbance}} - \tau_{\text{conditioning}}$) is smaller for higher conditioning shear stresses.

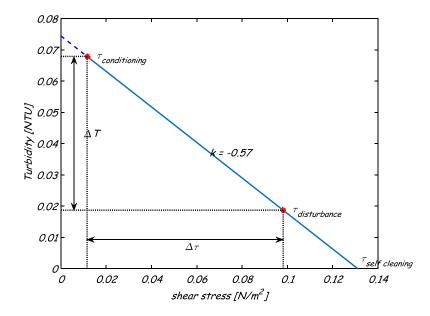


FIGURE 2. TURBIDITY POTENTIAL AS A FUNCTION OF THE SHEAR STRESS, ACCORDING TO THE PODDS MODEL. WITH THE GIVEN VALUES OF CONDITIONING, SELF-CLEANING AND DISTURBANCE SHEAR STRESS, 72.5% OF THE POTENTIAL TURBIDITY WOULD BE SUSPENDED.

2.4 Biofilm detachment: type and likelihood of disturbances

The likelihood of a hydraulic event, and the corresponding size of the disturbance (excess shear stress), depend on the type of event, and are not equal over the entire DWDS. Various types of events and their likelihood are discussed below.

<u>Pipe breaks</u>: The probability of a pipe break depends on pipe diameter, pipe material and year of installation. It may also depend on the surroundings: stresses from ground movement, traffic, etc. The Dutch pipe failure registration database USTORE (Vreeburg et al. 2013) can provide input for the pipe specific failure probability. In trunk mains $\tau_{\text{self-cleaning}}$ is relatively high and may not be easily exceeded during normal (or conditioning) operations, nor during disturbances. In the distribution part of the DWDS it seems to be very likely that a pipe break will lead to disturbance shear stresses above the self-cleaning shear stress, thus implying that a maximum biofilm exchange of 100% is possible. Under self-cleaning

conditions during normal operation the fraction of biofilm detachment will be 0. The biofilm detachment risk (BDR) due to pipe breaks would thus be determined by the pipe break frequency of the surrounding pipes multiplied by 0/1 depending on the conditioning shear stress. The pipe break frequency typically ranges from 0.017 per km per year for 50 mm PVC pipes to 0.123 per km per year for 100 mm Cast Iron pipes (from USTORE). We may assume that a pipe break typically has a maximum effect over a range of 100 m. For the BDR model it thus will be assumed that due to pipe breaks each pipe has a 0.2 to 1.2 % chance per year of experiencing a maximum biofilm exchange, compensating for specific local pipe break frequencies.

Use of hydrant: A large change in flow can occur from the use of hydrants in case of a fire or (controlled) flushing actions. A rough estimate of maximum hydrant use in the Dutch DWDS is that each pipe is affected once per year with a relatively low disturbance and once per year with a relatively high disturbance due to flushing or firefighting (Blokker et al. 2013). How this varies over the DWDS was not assessed. In the Netherlands the fire department does not register which hydrants are used in fire control, nor for assessment of hydrant functionality. One number we found was a frequency of use of 6.45% for a specific area in the Netherlands (Blokker et al. 2007a). There is some information on the location of fires, but it is unclear if the water in the truck was enough or a hydrant was required to extinguish the fire. Most fires are found in city centres. It is unclear if this can be explained by a higher population density or higher density of older (less fire resistant) buildings, or something else, and thus if there is a correlation with properties of the DWDS, such as pipe length and diameter. Also, the fire department does not monitor the flow from the hydrants. A hydraulic network model can help to determine the maximum flows. It is likely that these maximum flows (30, 60, 90 m³/h) will lead to exceedance of the self-cleaning shear stresses, and thus lead to 100% of the maximum biofilm exchange. Dutch water companies typically flush their network every four to five years, using one out of three to eight hydrants per flushing action. This means a hydrant has a probability of use of 3 to 8%. And each pipe has a probability of being flushed of 20 to 25%. Most water companies do have a registration of flushing plans, including hydrant ID's and required flows, where the flows have been checked with a hydraulic network model. For flushing the aim is to apply a $au_{ ext{disturbance}}$ that is high enough to suspend 100% of the potential discolouration material. As the aim of flushing is to remove the material in a controlled way, this does not add to the BDR. For the BDR model it will be assumed that due to uncontrolled hydrant use each pipe has a 5% chance per year of experiencing a maximum biofilm exchange, without taking into account specific local circumstances.

Valve closure: Closing a valve in the DWDS may lead to a change in contact times, a change in flow direction, a change in flow velocity. The probability of closing a valve can be estimated from the work that is done on networks. The valve closure for pipe repair and flushing will not be taken into account, as the change in flow due to pipe break and flushing respectively have already been taken into account. This means that only valve closure for planned maintenance should be considered. With a maximum of once per 100 years that a section will be closed for maintenance and three valves per section, each valve has a probability of use of 3%. The effect on the pipes within the section is not considered as after maintenance the section is being flushed. The effect on the pipes outside of the section, with that section not being supplied during maintenance, is typically a decrease in flow velocity in the pipes near the valves. This, in the PODDS concept, does not lead to suspension of discolouration material or removal of biofilm. After the work is finished some of these valves may unintentionally be left closed. This means that the setting of valves is not only of interest with respect to the hydraulic disturbance, but also for the determination of the steady state biofilm under "normal" operations. An inventory of one of the Dutch water

companies of the status (open or closed) of a statistically significant amount of valves showed that ca. 0.7% of the valves are not set to the expected position (Mesman 2016). For the BDR model it will be assumed that due to valve closure there is no extra BDR.

<u>Change in demand</u>: A large change in demand can occur from high coinciding residential demands e.g. during the interim of the finals of the world cup football or incidental hot days. The probability of such events is difficult to assess, but in the Netherlands seems to occur only once per year on average. The likelihood of coinciding demands is larger at a smaller spatial scale, where a small amount of homes is being supplied, and thus has a local effect. For the BDR model it will be assumed that due to seasonal peaks in demand there is limited extra BDR.

2.5 Modelling biofilm detachment risk in the DWDS

The BDR is related to the effect and to the likelihood of a given hydraulic disturbance. Overall, for the BDR model it will be assumed that due to hydraulic events

- each self-cleaning pipe has a no risk of biofilm exchange (BDR = 0);
- all other pipes experience a maximum biofilm exchange (which is not equal to total biofilm detachment) with 5% chance due to uncontrolled hydrant use plus a 0.2 to 1.2% chance depending on the burst probability of the surrounding pipes.

The BDR effect can be described from Eqs. (12), (15) and (18), and we find for non-self-cleaning pipes and self-cleaning pipes respectively:

$$C_B = \frac{4\phi(C_S - C_{S,min})}{D \cdot \mu_m} \approx \frac{4\phi C_S}{D \cdot \mu_m}$$
 (19a)

$$C_B = 0 ag{19b}$$

The BDR thus depends on

- pipe diameter directly;
- burst probability of the surrounding pipes, and thus depends on pipe material and diameter (as follows from USTORE);
- maximum daily flow velocities, and thus with a given water demand and network layout depends on pipe diameter.

3 Discussion

3.1 Hydraulic circumstances in DWDS

To be able to get a sense of how the model would work on a real DWDS, some calculation were done on a network in Purmerend. This network is described in more detail in (Blokker et al. 2010). It serves ca. 2000 homes through 12.3 km of AC and PVC pipes.

The example network is not designed according to the self-cleaning design rules, therefor it is assumed that 100% of the available biofilm is detached with a large hydraulic disturbance. An analysis of maximum flow velocities and shear stresses during normal operation shows that there are some pipes that experience shear stresses above 0.15 N.m⁻² (Figure 3, Figure 4).

The assumption that in the distribution part of the DWDS a pipe break will lead to disturbance shear stresses above the self-cleaning shear stress (§ 2.4) needs to be tested. This can be done with a hydraulic network model where pipe breaks are simulated using the emitter function in EPANET. This may show that pipes at the end of the network may lead to smaller excess shear stresses as less pressure is available.

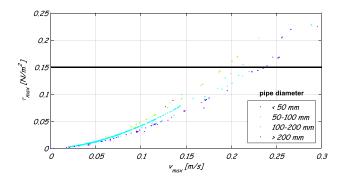


FIGURE 3. MAXIMUM FLOW VELOCITIES AND SHEAR STRESS DURING NORMAL OPERATION IN PURMEREND AREA A. INDICATED IN BLACK IS THE SELF-CLEANING SHEAR STRESS OF $0.15\ N/m^2$.

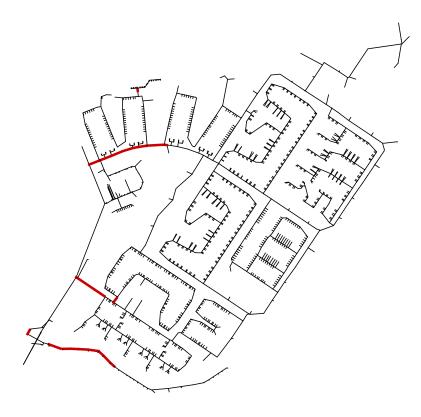


FIGURE 4. PURMEREND AREA A; IN RED THE PIPES WHICH EXPERIENCE $\tau_{\rm conditioning} > 0.15 \ {\rm N/m^2}.$

3.2 Effect of diameter

From § 2.5 we find that the pipe diameter influences the BDR directly through the pipe volume, and indirectly through the burst rate and self-cleaning capacity. Smaller pipe diameters would leak to larger burst rates and thus a higher probability of biofilm detachment, and to smaller volumes and thus a higher effect of biofilm detachment. At the same time smaller pipe diameters are more likely to be self-cleaning and self-cleaning pipes have a BDR of (near) zero.

3.3 Effect of temperature

The BDR through Eq. (19a) and Eq. (8) is temperature dependent. At higher temperatures the maintenance rate is higher (exponential increase), while the diffusivity is also higher (but with most likely only a linear increase), and thus with the same substrate concentration, the biofilm density that can be maintained is lower. An increase in temperature (seasonal effect) would thus lead to biofilm decay and detachment. If this biomass would then be transported in suspension or bed load transport and accumulate with the sediment, an increase in temperature could lead to an increase in discolouration risk. An increase in discolouration risk with increase in temperature has been observed, but not yet explained (Mounce et al. 2016; van Summeren et al. 2015). This of course depend on how large the temperature effect is on maintenance and thus biofilm decay. This needs to be studied further.

Also, there may be an effect of temperature on substrate concentration from the WTW, which would also a variation in substrate uptake over the year. Not only total substrate, but also substrate composition may change over the year. This could also affect substrate uptake and biofilm maintenance.

Furthermore, local hotspots, e.g. due to district heating systems, may lead to a steady state biofilm at that location which differs from the surrounding area. This would then mean that hotspots may cause a spatial variation in biofilm density.

3.4 Risk reduction

Risk reduction measures for biofilm detachment are partly similar to risk reduction measures for discolouration:

- Reduce incoming biomass (substrate), similar to reduction of particle load;
- Flushing pipes is not a practical solution to reduce BDR as within 100 days there is a stable biofilm;
- Self-cleaning networks should also reduce the BDR

The self-cleaning network concept for discolouration has been shown to work in the tertiary network. If this concept would also hold in the secondary network needs to be explored. If a self-cleaning network is effective in reducing the BDR also needs to be investigated.

3.5 Validation of assumptions

The BDR model as we have described it, is based on several assumption that need to be validated. One approach is a further in-depth literature review, another approach is doing lab and field measurements. We propose the following next steps:

Assumption 1: biofilm density is a function of source concentration (Eqs. (9), (12)). Test the assumption (literature) and determine typical values (literature and lab tests) for Dutch DWDS (non-chlorinated, with biologically stable water). Furthermore it is recommended to test if parameter values depend on temperature, substrate type, flow velocity or shear stress, etc. (§ 3.6).

<u>Assumption 2</u>: depending on the conditioning shear stress, the disturbance shear stress and the PODDS "maximum shear stress" which we assume is equal to the self-cleaning shear stress, more or less biofilm is detached (Figure 2). This assumption should be further substantiated to hold in the tertiary, secondary and primary part of the DWDS.

- Flushing experiments in the DWDS show that the flushing water does contain ATP, suggesting biomass is being detached. However, these experiments also show that most of the biofilm cannot be removed with flushing alone. It is unclear how much of the biofilm (which would be modelled in the steady state biofilm model) is equal to 100% of the "detachable" biofilm.
- It is not yet known if the self-cleaning velocity, which keeps pipes from fouling, is the same as the PODDS "maximum shear stress" above which no further turbidity increase is found
- The values for the self-cleaning shear stress should be determined for more than the small diameter (<200 mm) AC and PVC pipes that have been studied so far.
- Also, we need to find out how the self-cleaning shear stress depends on the biofilm EPS, and e.g. if there is an influence of chlorination.

Something to concern in this respect is that it has been shown that the tertiary network that is designed according to the self-cleaning design principles, indeed has no (or a very low) discolouration risk (Blokker et al. 2007b). However, there does seem to be a steady state biofilm in those networks (Blokker 2015). Biofilm can be attached to the pipe wall, and to the sediment on the wall (measurements show ATP varies between 90% on the wall / 10% on sediments to 40% on wall / 60% on sediments). The BDR may be different for biofilm on the wall and on sediments.

Assumption 3: the BDR varies over the DWDS mostly because of the probability of disturbances varying over the DWDS (also a consequence of assumptions 1 and 2, § 2.4). There is no modelling approach described in the literature yet how probabilities of pipe bursts would lead to hydraulic disturbances in the surrounding pipes. This does seem to be possible, but it should be discussed if this is a good approach. For discolouration risk, this particular contribution to the risk was discarded. It was assumed that at all locations the disturbance potential was larger than zero, and the relative potential was never taken into account.

<u>Consequence 1</u>: diameter is the most important factor in BDR. Determine for some example networks how this works out (§ 3.2).

<u>Consequence 2</u>: temperature increase would lead to biofilm density decrease and thus biomass concentration increase in the water (§ 3.3).

3.6 Outlook: planned laboratory experiments and model studies

At KWR a comparable test rig as the one at the University of Sheffield (UoS) is being build, but with a different pipe diameter (100 mm, instead of 80 mm at UoS), different pipe material (PVC, instead of PE at UoS) and continuous refreshing flows (instead of a looped system at UoS), and without the use of chlorine (with chlorine at UoS). Kimberly Learbuch will do experiments, within the BTO, in this system that are aiming to study how exchange of biomass between water and biofilm in a pipe is affected by flow variations.

Isabel Douterlo has won a grant from the UK ESPRC to work on biofilm in DWDS through doing controlled measurements at the pipe rig at UoS. She will measure biofilm density and genomics under various circumstances, such as variable temperatures (16, 8, 16, 24 °C), various levels of chlorine concentrations, and various phosphate concentrations. During prior research at the pipe rig Rebecca Sharp, Katherine Fish and Isabel Douterlo have developed test methods and looked at the effect of different shear stress patterns (constant, low variable, and high variable), limited effect of temperature (only at 8 and 16 °C) and limited effect of chlorination. The results have not been published yet.

In the BTO of 2018 it is expected that a discolouration prediction tool will be build, based on earlier research (van Summeren and Blokker 2016). Once this tool has been built and validated, and lab tests have been performed to determine parameter values, it may be an interesting option to add the microbial risk model onto the discolouration prediction tool.

Once the model assumptions have been further substantiated the model can be developed further. It will then need to be used to identify which processes are the most important and, as a result, need to be developed in more detail. Such a sensitivity analysis can be done by applying the model on a real DWDS. In addition, by applying the model to a real DWDS, several scenarios can be tested, such as the effect of increasing temperatures or decrease in AOC levels.

It is recommended to also use this model to determine, and validate, where in the DWDS the highest biofilm density can be found, if and how AOC decreases over the length of the DWDS (and thus determine values for ϕ), what the effect is of growth enhancing materials (e.g. in pipe materials). A future step would be to look at specific microorganisms in the biofilm.

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I. Quantifying biofilm density

Rittmann and McCarty (1980) describe how the biofilm density is maintained by a substrate flux (*J*, mg_{c.s}.m⁻².day⁻¹), which is affected by the substrate concentration in the water. In this section an attempt is made to determine from the literature how several parameters correlate and what typical values are. So far, this attempt is incomplete, and needs to be extended. Not just based on the available literature, but also with some more lab tests.

If we try to determine some of the parameter values from the literature for a specific situation, we find that $C_{\text{S}_{\min}}$ is given by (Rittmann and McCarty 1980):

$$C_{S,min} = K_S \cdot \frac{r}{Y \cdot k - r} \tag{20}$$

with Y is yield = 0.5 $\text{mg}_{\text{C,B}}$. $\text{mg}_{\text{C,S}}^{-1}$, r is decay rate (day⁻¹), K_{s} is the half-velocity coefficient (10⁴ $\text{mg}_{\text{C,B}}$. m^{-3}) and k is maximum specific rate of utilization (8 $\text{mg}_{\text{C,S}}$. $\text{mg}_{-1\text{C,B}}$.day⁻¹). (Yuanxiang and GOVIND 2008) give for the maximum specific utilization rate 6.day⁻¹, for the substrate half-saturation concentration 4g.m⁻³, and for the true Yield 0.63 g/g.

We will assume that $r/Y = M_c$; it is uncertain if this is allowed, but this would lead to

$$C_{S,min} = K_S \cdot \frac{M_S}{k - M_S} \approx \frac{1.10^4}{8} \left[\frac{day}{mg. m^3} \right] M_S$$
 (21)

 C_{sdeen} is given by:

$$C_{S,deep} = 4.6C_{S,min} (1 + \sqrt{2} \cdot D_f^* \cdot L^*)$$
 (22)

with L^* the relative biofilm layer depth (= L/τ), with τ the standard biofilm depth, L the depth of effective diffusion layer (in m), D_f^* the relative diffusivity of substrate in biofilm compared to water (typically 0.8, (Rittmann and McCarty 1980), 0.2 to 0.8 (Stewart et al. 2001)). And

$$\tau = \sqrt{\frac{2K_sD_f}{k \cdot X_f}} \tag{23}$$

with $X_{\rm f}$ the cell concentration within the biofilm (mg.m⁻³). Using typical values of $K_{\rm s}=10^4$ mg_{C,B}·m⁻³, k=8 mg_{C,S}·mg_{-1C,B}·day⁻¹, $D_{\rm f}=6.4$ 10⁻⁵ m².day⁻¹ and $X_{\rm f}=40$.10⁶ mg.m⁻³ leads to $\tau=63$ 10⁻⁶ m. A web search lead to values of $X_{\rm f}=5$, 12 and 20 mg.l⁻¹ and $\tau=77$ 10⁻⁶ m (Stewart et al. 2001). A circular reasoning seems to occur here, since $X_{\rm f} \cdot L=\rho_S$.

 J_{deep} can be described with:

$$J_{deep} = C_{S,deep} \cdot \sqrt{2} \cdot \frac{D_f}{\tau} = 4.6C_{S,min} \left(1 + \sqrt{2} \cdot D_f^* \cdot L^* \right) \cdot \sqrt{2} \cdot \frac{D_f}{\tau}$$
 (24)

The value of ϕ can thus be determined:

$$\phi = \frac{J_{deep}}{C_{S,deep} - C_{S,min}} = \frac{4.6(1 + \sqrt{2} \cdot D_f^* \cdot L^*)C_{S,min} \cdot \sqrt{2} \cdot \frac{D_f}{\tau}}{4.6(1 + \sqrt{2} \cdot D_f^* \cdot L^*)C_{S,min} - C_{S,min}}$$

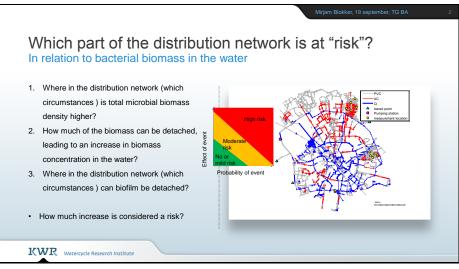
$$= \begin{cases} \frac{4.6\sqrt{2}D_f}{3.6\tau} = 1.8 \frac{D_f}{\tau} & \text{if } L^* \ll 1 \\ \frac{\sqrt{2}D_f}{\tau} = 1.4 \frac{D_f}{\tau} & \text{if } L^* > 1 \end{cases}$$
(25)

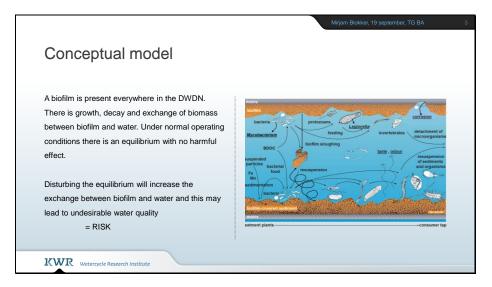
with D_f the molecular diffusivity of substrate in biofilm (typically 6.4 10^{-5} m².day¹, (Rittmann and McCarty 1980)). This means ϕ is equal to 1.4 to 1.8 m.day¹, and ϕ = 1.6 m.day¹ for $L = \tau$ (values of 1000 μ m, 10 cells thick mentioned by Stewart et al. (2001)).

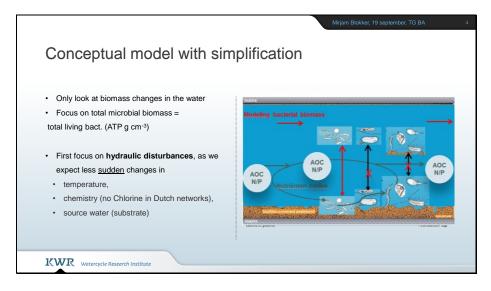
This results in values of 0.45 mg.cm⁻², where in their table Rittman and McCarty show 0.25 mg.cm⁻². This means that probably Eq. (25) is not correct. Maybe some other of their publications (Rittman and McCarty 1981; Rittmann and McCarty 1978) will give more insight into this.

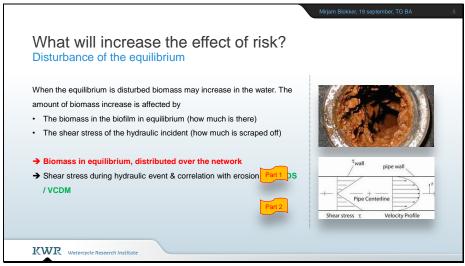
II. Presentation 19 September 2017



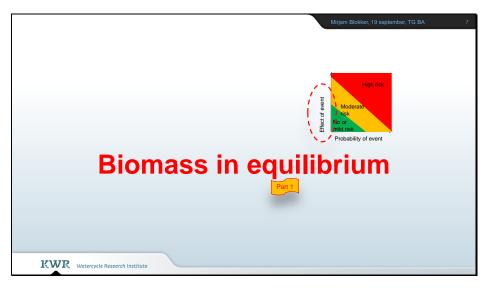


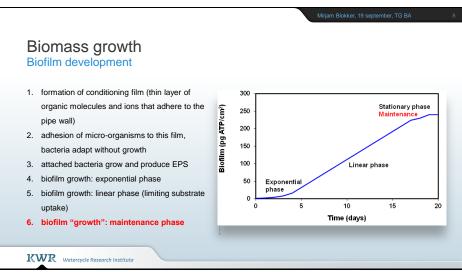












Biofilm maintenance phase No net growth, but processes do take place Research in non-chlorinated DWDS has shown: 1. micro-organisms in the biofilm decay and their biomass 1. negligible: after 200 days still all cells viable in biofilm is replaced by microbial growth, (Boe-Hansen et al., 2003) 2. protozoans graze on the biofilm and bacterial loss by 2. Negligible: protozoans can gain just enough energy to grazing is complemented by bacterial growth, maintain their biomass in the biofilm maintenance phase (van Lieverloo et al. 2002 and references therein) 3. biofilm is released in the water and complemented by 3. Not dominant: the bacterial communities and in biofilms on pipe walls are different, so "no" exchange (Liu et al. 4. micro-organisms gain just enough energy from the low nutrient concentrations in drinking water environment to 2014; Roeselers et al. 2015) run their cell maintenance processes (i.e. cell 4. Most dominant KWR Watercycle Research Institute

Modelling respiration (1)

Concept

Substrate from the water is used to maintain the biofilm, substrate uptake ($S_{\rm u}$, mg.m $^{\rm -3}$) can be described as:

$$S_u = \frac{M_S \cdot B \cdot \Delta t}{V}$$

with B the biofilm mass (mg), Δt the contact / reaction time (day), $\,V\,$ the water volume (m³) and $\,M_{\rm S}$ the maintenance coefficient (mg substrate-C mg biomass-C⁻¹ day⁻¹), the amount of substrate needed per day to maintain a certain biomass.

The substrate reaches into the biofilm through molecular diffusion. This means that the substrate concentration (S, mg.m-3) drives the amount of substrate that can reach the cells in the biofilm and thus how much substrate is available for maintenance of certain biofilm density.

There is a minimum S needed.



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Modelling respiration (2) In equations

The substrate uptake (S_u, mg.m⁻³) per time can be

$$\frac{S_u}{\Delta t} = \frac{4M_S}{D}$$

with $b = B/(\pi DL)$ the biofilm density (mg.m⁻²), D the pipe diameter (m) and L its length (m).

Diffusion means that the substrate flux (required for biofilm maintenance) is linearly related to the substrate concentration (S), and $S_{\rm u}$ can be described as

(Rittman and McCarty 1980):
$$\frac{S_u}{\Delta t} = \frac{4\phi(S-S_{min})}{D}$$

$$S(t) = S_{min} + (S(0)-S_{min})e^{\frac{-4\phi}{D}t}$$

with ϕ a conversion factor (m.day⁻¹).

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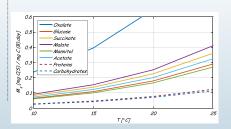
Modelling respiration (3) In numerical values

Measurements in the Netherlands show there is no measurable decrease in AOC over the length of the DWDS: S is equal over the entire DWDS, and thus b is equal over the DWDS with no dependence on diameter or flow velocity: $b = \frac{\phi(S - S_{min})}{M}$

$$b = \frac{\dot{\phi}(S - S_{min})}{M_S}$$

 ϕ depends on molecular diffusivity (substrate type), and the standard biofilm depth (related to substrate type and biofilm density). Values indirectly deferred (0.11 mm.day⁻¹ ???)

 $M_{\rm s}$ depends on the type of substrate, and on temperature



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