Elucidation and control of biofilm formation processes in water treatment and distribution using the unified biofilm approach

D. van der Kooij, J.S. Vrouwenvelder and H.R. Veenendaal
Kiwa Water Research, PO Box 1072, 3430 BB Nieuwegein, The Netherlands

Abstract Controlling biological processes in water treatment and distribution is a major challenge to water supply companies. In the Netherlands, the use of chlorine-based disinfectants in water treatment is limited as much as possible and treated water is distributed without disinfectant residual in most cases. Biofilm formation processes in water treatment and distribution are studied using adenosinetriphosphate (ATP) as the parameter for active biomass. ATP measurements are applied to assess biofilm concentrations in distribution systems, in the biofilm monitor to determine the biofilm formation rate of treated water, in the biomass production potential test to determine the effect of pipe materials on microbial growth and in membrane systems to quantify biofouling. The use of a single parameter enables to compare biofilm concentrations in all situations and contributes to the understanding and control of biofilm formation processes in water treatment and distribution. This approach has been designated as the Unified Biofilm Approach.

Keywords Adenosinetriphosphate; biofilm formation rate; biofilms; biological stability; biomass production potential; unified biofilm approach

Introduction
Biofilms play an important role in water treatment and distribution. During soil passage and in filtration processes, biofilms contribute to the removal of organic and inorganic biodegradable compounds and also play a role in the removal of pathogens. Under certain conditions however, biofilm formation seriously hampers treatment process, e.g. biofouling of membranes and clogging of filter beds (Ridgway and Flemming, 1996; Hijnen and van der Kooij, 1992). Also abstraction wells or recharge wells may become clogged due to biological processes (Kawanishi et al., 1990). In water distribution, excessive biofilm formation leads to a deterioration of the microbiological quality of treated water. Major disadvantages include: (i), regrowth of coliforms (LeChevallier et al., 1987); (ii), multiplication of opportunistic pathogens such as Mycobacterium spp., Legionella spp., Aeromonas spp. and Pseudomonas spp. (Engel et al., 1980; Havelaar et al., 1990; Tobin et al., 1980; Von Reyn et al., 1994). Legionella especially multiply at elevated temperatures in plumbing systems (Wadowsky et al., 1982). Other problems include increased heterotrophic plate counts, complaints about invertebrates (van Lieverloo et al., 1994), color, taste and odor and microbially induced corrosion (MIC) (Lee et al., 1995). Controlling biofilm formation is therefore a precondition in all stages of water supply, aiming at optimal use on one side and effective limitation on the other. This is particularly the case in the Netherlands where biological processes are applied in water treatment, and treated water is distributed without a disinfectant residual. The aspect of biostability, which is related to both biofilms in water treatment and in distribution, has been studied for long period (van der Kooij et al., 1999).

Biofilm formation processes have attracted much attention in the past few decades, because water-exposed surfaces are the sites where microbiological activity in water systems is located. The formation of biofilms is caused by attachment of microorganisms to
surfaces followed by growth. Attachment processes have been studied in detail by many investigators and also the various stages of growth, viz. exponential growth, linear growth and stationary phase (Characklis, 1973; LaMotta, 1976). These phases are schematically presented in Figure 1. Elucidation of the factors determining these processes in the various phases requires techniques to determine the concentration and nature of the microorganisms contributing to biofilm formation. Parameters most commonly used to determine the concentration of attached biomass include: heterotrophic plate count (HPC) on various culture media and total direct cell count using epifluorescence microscopy. Typical biomass components such as proteins, carbohydrates and total biomass can be analyzed at higher biofilm concentrations. The parameters mentioned all have their typical advantages and limitations, e.g. HPC techniques only detect (an unknown fraction of the) culturable microorganisms whereas total cell counts and biomass parameters (proteins, carbohydrates) do not differentiate between dead and live organisms. The use of adenosinetriphosphate (ATP) as a biomass parameter has a number of advantages, including: (i), ATP, is an energy carrier in all active biomass and is not present in dead microorganisms (Holm-Hansen and Booth, 1966); (ii), ATP analysis is rapid and accurate, even at very low concentrations. For the ratio between cell carbon and ATP, generally a value of 250 is used (Karl, 1980; Holm-Hansen and Booth, 1966). The ATP concentration thus gives quantitative information about the concentration of active biomass, either attached or suspended. Attached active biomass reflects the response of the biofilm to the supply of energy sources, both organic and inorganic. Determining biofilm concentrations with ATP enables a simple comparison of biofilms in a variety of situations prevailing in water treatment and distribution, but also in other man-made and natural situations. This paper describes the use of ATP analysis for the quantitative assessment of biofilms in systems developed for monitoring the biofilm formation characteristics of treated water and materials and on surfaces exposed to water during treatment and distribution.

**Biological stability of treated water**

**AOC test.** Biofilm development is promoted by microbial utilization of biodegradable compounds, either originating from treated water or from the exposed material. AOC and BDOC tests are commonly used to assess the concentration of growth-promoting organic compounds present in water (van der Kooij et al., 1982; Joret and Levy, 1985). The AOC test is based on determining the maximum level of growth of two pure bacterial cultures inoculated in pasteurized samples of water to be tested. These water samples (600 ml) are incubated in thoroughly cleaned glass-stoppered Erlenmeyer flasks with a volume of 1 L. The test strains are *Pseudomonas fluorescens* strain P17, which is capable of utilizing a

![Figure 1](https://iwaponline.com/wst/article-pdf/47/5/83/422560/83.pdf)  
**Figure 1** Biomass concentration on a surface ("biofilm") exposed to flowing water (theoretical). ATP gives the concentration of active biomass
wide range of compounds at very low concentrations (van der Kooij et al., 1982a) and \textit{Spirillum sp.} strain NOX, which only utilizes carboxylic acids (van der Kooij and Hijnen, 1984). AOC concentrations are calculated from the maximum colony counts of these two strains grown as a mixed culture in the water samples using the yield values of these organisms for acetate. AOC concentrations in treated water in the Netherlands range from less than 10 \( \mu \text{g} \ \text{C L}^{-1} \) in slow sand filtrates and in groundwater supplies to values of about 50 \( \mu \text{g} \ \text{C L}^{-1} \) in surface water supplies with ozonation in water treatment (Van der Kooij, 1992). The AOC fraction utilized by strain P17 in the presence of strain NOX is usually less than a few \( \mu \text{g} \) of C L\(^{-1}\), indicating that the major fraction of AOC in treated water consists of carboxylic acids. AOC values below 10 \( \mu \text{g} \ \text{C L}^{-1} \) hardly decline in drinking water during distribution, indicating that AOC uptake is very limited at these low concentrations. Also HPC values do not increase at these concentrations and therefore an AOC level of 10 \( \mu \text{g} \ \text{C L}^{-1} \) has been derived as a reference value for biostable drinking water in which multiplication of bacteria contributing to HPC values is very limited.

\textbf{Biofilm formation rate (BFR).} Observations on increasing numbers of aeromonads in a number of groundwater supplies at AOC concentrations below 10 \( \mu \text{g} \ \text{C L}^{-1} \) revealed that the definition of biostable water had to be extended. Furthermore, AOC tests and also BDOC tests do not provide information about the rate of uptake nor about the effects of non-organic growth-promoting compounds, e.g. ammonia, sulfides. For these reasons, a simple tool, the biofilm monitor, had been developed to determine the biofilm formation characteristics (biofilm formation rate, BFR; biofilm formation potential, BFP) of drinking water (Van der Kooij et al., 1995). This biofilm monitor consists of a vertically-placed glass column containing glass cylinders (surface area: 17 cm\(^2\)) on top of each other. The water to be investigated is flowing through the column at a rate of 0.2 m/s. Biofilm formation is determined as a function of time by collecting the glass cylinders from the column at regular intervals and determining the biomass concentration on the surface of these cylinders. Attached biomass is released from the glass surface by a series of three low energy sonications, of 2 min each. Subsequently, the ATP concentration is determined in the obtained biomass suspension and the biofilm concentration is calculated. The BFR (pg ATP cm\(^{-2}\) d\(^{-1}\)) of the water can be calculated as the slope of the linear increase of the biofilm concentration with time (see Figure 1). The BFP (pg ATP cm\(^{-2}\)) of water is defined as the maximum biofilm concentration, but in many cases this value is not reached within the applied exposure period of 150 days. Figure 2 gives a typical example of biofilm formation in the biofilm monitor supplied with treated ground water (temperature: 12.5°C; DOC = 2.4 mg L\(^{-1}\); AOC = 5.0 \( \mu \text{g} \ \text{C L}^{-1}\)). Biofilm formation was determined with ATP analysis, total direct cell counts (TDC) and HPC values on R2A-medium (10 days incubation at 25°C). The BFR value as calculated from the linear increase of the ATP concentrations was 12.5 \( \pm \) 0.7 pg ATP cm\(^{-2}\) d\(^{-1}\). The biofilm concentration was about 1,850 pg ATP cm\(^{-2}\) after 150 days, but the maximum level had not been reached. TDC values reached a level of about 3 \( \times 10^7 \) cells cm\(^{-2}\). TDC values were proportional with the ATP values (Figure 2C) and an average ATP concentration of 7.4 \( \times 10^{-11}\) µg ATP per cell was calculated. This value is lower than the ATP content of bacteria grown as pure culture (Hamilton and Holm-Hansen, 1967) and may reflect the effect of the nutritional status of the bacteria growing in the biofilm. HPC values attained a maximum level of about 10\(^5\) CFU cm\(^{-2}\) and initially were about 1% of the TDC values, but this percentage had decreased to less than 0.5% after 150 days. These results demonstrate that different biomass parameters give different information about biofilm formation. For reasons given above, ATP had been selected as the parameter of choice for determining biofilm formation. BFR values in treated water in the Netherlands usually are below 30 pg ATP cm\(^{-2}\) d\(^{-1}\),
ranging from less that 1 pg ATP cm⁻² d⁻¹ for slow sand filtrates to about 100 pg ATP cm⁻² d⁻¹ for drinking water prepared from ground water containing ammonia and methane. Furthermore, a linear relationship has been observed between low concentrations of acetate-C added to drinking water and the BFR value. From this relationship a BFR value of 35 pg ATP cm⁻² d⁻¹ for 1 µg of added acetate-C L⁻¹ was obtained (van der Kooij et al., 1995a). Consequently, the BFR values observed for treated water correspond with concentrations of acetate-C (equivalents) which were clearly below 1 µg of C L⁻¹ in most cases. These calculated acetate-C concentrations were usually a small fraction of the AOC concentration, which was less than 10 µg C L⁻¹ in most types of treated water. Combining the results of the two methods gives a two dimensional scheme for defining the biological stability of drinking water, with the AOC concentration on the x-axis and the BFR-value on the y-axis.

Biofilm concentrations on pipe walls. Typical HPC values on surfaces range from less than 100 CFU cm⁻² to more than 10⁶ CFU cm⁻² in chlorinated supplies (LeChevallier et al., 1987) and values from 10³ to 10⁶ CFU cm⁻² were observed in an experimental reactor supplied with treated water (Volk and LeChevallier, 1999). Data about biofilm concentrations have also been determined in unchlorinated supplies in the Netherlands. For this purpose, segments of pipes of unplasticized PVC, which is the main (more than 40%) pipe material, have been collected from a series of water supplies and biofilm concentrations were determined using ATP analysis. Biofilm concentration on these surfaces ranged from less than 100 pg ATP cm⁻² to about 6000 pg ATP cm⁻² with a median value of 700 pg ATP cm⁻² (van der Kooij et al., 1999). The lowest biofilm concentration values were observed in supplies distributing aerobic groundwater with a low concentration of organic compounds and a low BFR value (<1 pg ATP cm⁻² d⁻¹). The highest biofilm concentrations were observed in supplies distributing drinking water derived from anaerobic groundwater containing ammonia and methane and BFR values greater than 50 pg ATP cm⁻² d⁻¹. These results demonstrate the effect of biostability (BFR values) on biofilm formation.

Biomass production potential (BPP) of materials in contact with treated water. Synthetic pipe materials in contact with treated water may enhance biofilm formation by releasing biodegradable compounds. Methods have been developed in the United Kingdom and in Germany to test the growth-promoting properties of such materials (Colbourne and Brown, 1979; Schoenen and Schöler, 1983). In the absence of a disinfectant residual high demands must be made on the biostability of materials. Therefore, a sensitive test based on the use of
ATP as biomass parameter has been developed to assess the BFP of materials (van der Kooij and Veenendaal, 1994). In this test material samples are incubated in biostable water (slow sand filtrate) at 25°C and the biomass concentration on the surface of these materials is determined as a function of time. More recently, this method has been adapted to the BPP method, where the BPP is defined as the sum of the BFP and the suspended biomass production (SBP). These parameters are expressed as pg ATP cm⁻² (van der Kooij and Veenendaal, 2001). The concentrations of active biomass on the material surface and in the water are determined with ATP analysis during a period up to 16 weeks. Initially, the concentrations of biofilm and suspended biomass are relatively high (depending on the type of material), but decrease to a stable level with most materials after about 50 days. Typical examples of such curves are presented in Figures 3 and 4. BPP values range from values less that 100 pg ATP cm⁻² (glass, stainless steel) to values greater than 10,000 pg ATP cm⁻² for plasticized PVC and natural rubber. Unplasticized PVC (PVCu) materials also have BPP values below 100 pg ATP cm⁻². Figure 4 demonstrates that maximum concentrations of attached biomass were reached within a few weeks on stainless steel (SS), PVCu and polyethylene (PE). Subsequently, the biofilm concentration declined rapidly and reached a

![Figure 3](image1.png)  
Fig. 3 Biomass production in the BPP test. A. Silicone rubber (■, attached biomass; □, attached and suspended biomass) and glass (closed circles: attached biomass; open circles, total biomass); B, plasticized PVC (■, attached biomass; □, attached and suspended biomass) and glass-fiber reinforced epoxy (●, attached biomass; ○, total biomass). BPP values are calculated as averages of the biomass concentrations in the period from 8 to 16 weeks

![Figure 4](image2.png)  
Fig. 4 Biofilm concentration on materials incubated in slow sand filtrate at 25°C (surface to volume ratio = 0.166 cm² cm⁻³) without replacement of the water
low and stable level with SS and PVCu after about 100 days. A different result was obtained with the PE material on which an increase of the biofilm concentration was observed after the decline phase. These observations suggest that a rapid utilization of biodegradable compounds present on the surface was followed by a slow utilization of compounds which either were also present on the surface or migrated to the surface.

The biofilm concentrations as observed with the SS and PVCu materials can be described by the following expression:

\[
BF_T = BF_0 \cdot e^{-kT} + BF_R
\]  

(1)

where \(BF_T\) is the biofilm concentration at time \(T\) (days) and \(BF_0\) is the (hypothetical) biofilm concentration at time 0. In reality, the concentration of biomass on the surface of the material is zero at day zero, but the biomass concentration increases rapidly within a few days with many materials. \(BF_R\) is the residual biofilm concentration, which may represent the biofilm formation potential in practice. The values of the characteristics as obtained from the experiment shown in Figure 4 are presented in Table 1. Biofilm formation on glass and PE did not follow Eq. (1), and for these materials the observed values are presented.

BFR levels < 100 pg ATP cm\(^{-2}\) are low in relation to the biofilm concentrations as observed on the surfaces of pipe materials. Hence: (i), the predominant pipe material used in the Netherlands has a high degree of biostability; and (ii), biofilm formation on this material in distribution systems is mainly caused by the utilization of biodegradable compounds from the water, confirming the observations with the biofilm monitor.

Inclusion of \textit{Legionella pneumophila} in BPP tests revealed a highly significant relationship between the BPP values and the extent of growth of \textit{Legionella} on a number of materials tested at 25°C (van der Kooij et al., 2002). Further research is needed to elucidate this relationship in more detail at incubation temperatures, which are more favorable for the growth of \textit{L. pneumophila}.

The use of different methods in different countries in Europe is hampering a European policy regarding testing and selecting materials in contact with treated water. In the framework of the development of the European Acceptance Scheme (EAS) for construction products in contact with drinking water (CDPW), a research project is being conducted aiming at harmonizing the test method for determining the microbial growth potential.

### Biofilm formation in water treatment

**Biofouling of membrane systems.** Biofouling is serious problem in the use of nanofiltration (NF) and reverse osmosis (RO) membranes in water treatment (Ridgway and Flemming, 1996). Assessment of the biofilm concentration in membrane elements by applying autopsy followed by ATP analysis revealed that biofilm concentrations on the membranes

<table>
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<tr>
<th>Table 1</th>
<th>Biofilm formation characteristics of materials as observed in the BFP test at 25°C</th>
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<tbody>
<tr>
<td>Material</td>
<td>(BF_0 \pm \text{s.d.} ) (pg ATP cm(^{-2}))</td>
</tr>
<tr>
<td>Glass</td>
<td>n.d. *</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>795 ± 138</td>
</tr>
<tr>
<td>Unplasticized PVC</td>
<td>1331 ± 82</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>1950 ± 70 (#)</td>
</tr>
</tbody>
</table>

*, n.d. not determined; **, observed value on day 362; #, value observed on day 7; ##, value observed on day 362
(including spacer) from a large number of installations ranged from less that 100 pg ATP cm$^{-2}$ to more than 10,000 pg ATP cm$^{-2}$. An increase of the pressure drop was generally observed at biofilm concentrations above 1000 pg ATP cm$^{-2}$. (Vrouwenvelder et al., 2000; Vrouwenvelder and van der Kooij, 2001). The rate of biofouling depends on the growth-promoting properties (AOC, BFR) of the feed water, which in turn depend on the composition of the raw water and the applied water treatment. Addition of an antiscalant can also affect biofouling when the added compound stimulates microbial growth. Testing of a series of antiscalants with AOC analysis and BFR measurements showed a wide range of growth promotion (Vrouwenvelder et al., 2000).

**Biological processes in filter beds.** AOC and BFR measurements are also used to investigate treatment processes. Concentrations of biomass in filter bed materials (sand, granular activated carbon) can be determined using ATP analysis (Magic and van der Kooij, 2002).

**Conclusions**

ATP is an attractive parameter for determining the concentration of active biomass (biofilm) on water exposed surfaces. It can also be used for determining the concentration of suspended biomass in treated water and in experimental systems. Collecting data with this parameter about biofilms as present in water treatment, in distribution systems, in biofilm monitoring devices and in materials testing gives a framework for evaluation of the observed concentrations. This framework has been designated as the Unified Biofilm Approach (van der Kooij et al., 1999). This approach is used in the Netherlands in combination with AOC and BFR measurements to elucidate microbiological processes in water treatment and distribution. The objectives are: (i), to control biofilm formation in water treatment; and (ii), to maintain biological stability in the distribution system.

**Acknowledgement**

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**References**


