BIOFILM FORMATION ON MATERIALS IN CONTACT WITH TREATED WATER

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Abstract
Materials releasing biodegradable compounds into drinking water can impair water quality by enhancing the multiplication of undesirable microorganisms, including opportunistic pathogens such as species of *Legionella*, *Mycobacterium* and *Pseudomonas,* respectively. Methods for testing the growth-promoting properties of materials have been developed in the UK (Mean Dissolved Oxygen difference test), in Germany (Slime Production test) and in the Netherlands (Biomass Production Potential test). In the BPP test adenosinetriphosphate (ATP) is used as the biomass parameter. The BPP value of unplasticized PVC, which comprises 50% of the distribution system pipes in the Netherlands, is less than 100 pg ATP cm^{-2}. This value is more than 100 times below the BPP value for plasticized PVC (PVCp). Calculations with data for MDOD and SP values reported for a selection of materials suggest that the criterion as defined for the SP test (0.1 ml/800 cm^2) is about 35 times more stringent than the criterion for MDOD (2.3 mg/l). These calculations also indicate that the BPP values as observed for PVCu (< 100 pg ATP cm^{-2}) are below the criterion for the SP value. Establishing criteria for the growth potential of materials require further investigations into the relation between growth potential and microbiological water quality problems in distribution systems and plumbing systems.

Regrowth problems and materials
Maintaining the quality of treated water during transportation, storage and distribution is a main objective in water supply. Biofilm formation in water distribution systems and plumbing systems may cause a deterioration of the microbiological quality of water used for consumption and other domestic purposes. Multiplication of microorganisms in these systems depends on a combination of conditions ('risk factors'), viz. (i) the concentration of biodegradable compounds, (ii) elevated temperature and (iii) residence time. Water temperature and residence time are difficult to control and limitation of regrowth therefore generally is achieved by maintaining a disinfectant residual in drinking water during distribution or by reducing the concentration of biodegradable compounds. Biodegradable compounds may originate from the raw water, water treatment (oxidation processes) or from sediments accumulating in the distribution system. Many observations in practice, in pilot plants and in laboratory tests have shown that also certain materials in contact with treated water can release compounds promoting the growth of microorganisms.

Frequently observed water quality problems caused by the multiplication of microorganisms in distributions and plumbing systems are:
- High heterotrophic plate counts (HPC). Elevated HPC values, exceeding criteria as defined in national legislation, is the most commonly reported signal of a deterioration of the microbiological water quality in water supply systems. The utilization of very low concentrations of biodegradable compounds present in treated water is a major cause of such multiplication ('regrowth'). The release of biodegradable solvents from

coatings has been identified as a cause of the increase of HPC values in storage tanks, reservoirs and pipes (1-4);

- Presence of coliforms. Coliform noncompliance has frequently been reported, even in water supply systems with a disinfectant residual (5, 6). Coliform bacteria can multiply at substrate concentrations as low as a few micrograms per liter (7, 8). These compounds may either originate from the water or from materials. Certain materials e.g. wood, coatings, lubricants have been found to enhance the growth of coliform bacteria (9, 10, 11). Also sediments and interactions between organic compounds in the water and the surface of corroding pipes may enhance coliform growth (12, 13);
- Presence of opportunistic pathogens including *Legionella pneumophila, Pseudomonas aeruginosa and Mycobacterium* spp.. Exposure to elevated levels of these bacteria in water pose a serious health threat, especially for immunocompromised persons. In 1980, *Legionella* present in warm tap water was identified as the etiological agent for waterborne cases of pneumonia (14). The annual number of cases of pneumonia may reach 20 per million persons (15). *Legionella* multiplies in certain protozoa grazing on biofilm bacteria (16, 17). Rubber components, other elastomers and plastic materials promote the multiplication of *Legionella* by enhancing biofilm formation (18, 19, 20, 21). *P. aeruginosa* also is an etiological agent for waterborne infections, mainly nosocomial pneumonia. An estimated 1400 death occur each year in the United States as a result of waterborne nosocomial pneumoniae caused by *P. aeruginosa* (22). The organism can multiply at very low concentrations of biodegradable compounds in water (23). Certain *Mycobacterium* spp. (*avium comple*x) are infectious for AIDS patients (24). Also mycobacteria can multiply in biofilm on material surfaces (25).
- Aesthetic problems. Microbial growth can cause esthetic problems, e.g. the presence of flocs, turbidity or impair the taste and odor of the water (26, 27).

Maintaining a disinfectant residual in drinking water during distribution is a commonly applied preventive measure. However, the formation of undesirable byproducts and complaints about taste and odor in relation to the presence of disinfectants stimulate limitation of the growth potential of water and materials as alternative (additional) measures to control regrowth. In the past two decades methods have been developed for determining the growth promoting properties of treated water, e.g. assimilable organic carbon (AOC), biodegradable dissolved organic carbon (BDOC). European and national legislation demands that materials in contact with tap water should not reduce the protection of human health as provided for in the Council Directive 98/83/EC (28). Consequently, given the potential of certain materials to enhance the growth of undesirable microorganisms and the steadily increasing number of synthetic materials, methods and criteria are needed to select materials on the basis of their growth-promoting properties.

This paper briefly describes such methods, which have been developed in Europe, with emphasis on the method developed in the Netherlands.

Methods for determining the microbial growth potential of materials

The Mean Oxygen Difference (MDOD) test as developed by Colbourne and Brown (29) is the standard method in the UK (30). The Slime Production (SP) test as developed by Schoenen (4) is the standard method in Germany (31). In the Netherlands, the BPP test has been developed (32, 33). The general characteristics of these tests are presented in Table 1.

Table 1 Characteristics of methods for determining the growth potential of materials in contact with drinking water

Condition/parameter	***W270***	***MDOD***	***BPP***
Temperature (°C)	Ambient (> 6°C)	30	25
Surface (S) of material (cm^2)	800	150	12 x 8
Volume (V) of water (cm^3)	100,000*	1000	600
S/V (cm^{-1})	n.a.**	0.15	0.16
Replacement	Continuously	Two times in a week	none
Water type	Tap water#	Tap water#	SSF##
Duration (weeks)	26	7.5	16
Microbiological activity	Slime volume	Oxygen	ATP

*, continuous flow (20 l/h); **, n.a., not applicable; #, dechlorinated (by applying GAC filtration) when needed; ##, SSF, slow sand filtrate

Mean Oxygen Difference (MDOD) test

In the MDOD test the additional oxygen consumption in the presence of the material to be tested is used as a parameter for microbiological activity. This method has been used for several decades and a large number of materials has been tested (27, 34). Typical MDOD values range from about 0.5 mg/l (blank with glass) to values of about 8 mg/l for paraffin wax (positive control). Materials with an MDOD value > 2.3 mg l^{-1} are considered not to be suited for use in contact with tap water (27,34).

Slime Production (SP) test

The Slime Production (SP) test as developed in Germany has also been used for a long time (4). The volume of slime on the surface of the tested material is used as the biomass parameter. This method is applied in a flow through (dynamic) system, with sheets of materials in contact with continuously flowing tap water. Typical SP values range from less than 0.1 ml (stainless steel blank) to more than 15 ml/800 cm^2 on solvent- containing bitumen or plasticized PVC (PVCp) (4). Materials with an SP value exceeding 0.1 ml/800 cm^2 are considered unsuitable in contact with drinking water (31).

The MDOD and SP tests have been compared on a number of PVC materials (35). Apart from one material, the test results in combination with the defined criterion gave the same conclusion regarding pass/failure of the tested materials (cf. Fig.4; see **Criteria**).

The Biomass Production Potential (BPP) Test

The BPP test was derived from the Biofilm Formation Potential test (32,33). In these tests Adenosinetriphosphate (ATP) is used as parameter for active biomass. ATP is an energy-rich compound, which is present in all living (= active) organisms. ATP analysis enables the detection of very low concentrations of microorganisms. The detection limit at directly application in (tap) water is 1 ng/l. Furthermore, the analysis can be conducted within a few minutes. ATP analysis is also used for determining the biofilm concentration on walls of distribution system pipes and in the biofilm monitor for determining the Biofilm Formation Rate (BFR) values of treated water (36,37). In this way a database of information about the biomass concentration in water, in biofilms and on materials is obtained, which facilitates the interpretation of individual measurements (Unifying Biofilm Approach).

In the BPP test the production of active biomass (ATP) as a function of time in the presence of the material to be tested. The BPP test is carried out as a static test, i.e. the water is not replaced in the course of the test. The analytical procedure includes the following steps:

- Representative samples (12 pieces of material with a total surface of about 100 cm^2) of the material are placed in 600 ml of biologically stable water (slow sand filtrate) in thoroughly cleaned glass-stoppered Erlenmeyer flasks (volume of 1 liter). The surface to volume ratio ($S\ V^{-1}$) is 0.17 cm^{-1};
- Inorganic nutrients (N and P) are added to prevent nutrient limitation and 5 ml of river water (filtered over a filter with pores of 1.2 μm) is added to ensure the presence of a large variety of bacteria;
- Samples of glass and silicone rubber are included as controls;
- Duplicate flasks are incubated in the dark at 25°C during 16 weeks;
- Periodically, samples of water and material are taken from the flasks for analysis of ATP. In the initial procedure (Biofilm Formation Potential test) samples of material were taken from flask A and water was sampled from flask B with a constant $S\ V^{-1}$ ratio. In the present procedure (BPP test) the S/V ratio is kept constant in both flasks by sampling volumes of water of 50 ml in combination with materials sampling;
- Biomass is removed from the material samples by applying a series of 6 sonications of 2 min each, using a water bath sonifier;
- The Biomass Production (BP, pg ATP/cm^2) is calculated from the concentration of attached biomass (biofilm) and the concentration of planktonic (suspended) biomass using the SV^{-1} ratio. The maximum biomass production (BP_{max}) is the maximum BP value which usually is observed within 2 weeks of incubation. The BPP value is the average of the BP-values obtained on day 56, 84 and 112. This BPP value is composed of the Biofilm Formation Potential (BFP) and the potential to form suspended biomass (SBP). Hence, BPP = BFP + SBP. These parameters are all expressed

as the quantity of active biomass per surface unit of the tested material (pg ATP cm^{-2}).

Legionella bacteria (or other selected bacteria) can be added to the test water at the start of the test. In this way also information is obtained on the degree to which a particular material is able to enhance the growth of *Legionella* (or other bacteria) under the test conditions (33, 38).

Growth-promoting properties of pipe materials used in the Netherlands

Distribution of drinking water in the Netherlands

Drinking water in the Netherlands is produced from groundwater sources (2/3) and surface water sources (1/3) respectively, to a total annual volume of about 1.2 x 10^9 m^3. Groundwater is treated with aeration and filtration processes and subsequently is distributed without chemical disinfection. In surface-water treatment the multibarrier concept is applied to remove organic and inorganic contaminants, pathogenic microorganisms and biodegradable compounds, respectively. Treatment consists of combinations of the following processes: storage in reservoirs, soil passage, coagulation/sedimentation, dual media filtration, ozonation, granular activated carbon filtration, slow sand filtration. Application of membrane filtration processes (ultrafiltration, nanofiltration, revere osmosis) and UV disinfection is limited to a few supplies, but these processes will also be incorporated in the multibarrier concept in the near future. In a few surface-water supplies, a low concentration of a disinfectant (chlorine dioxide or chlorine) is added to the water leaving the treatment facility to reduce HPC values in the (GAC) filtrates (36).

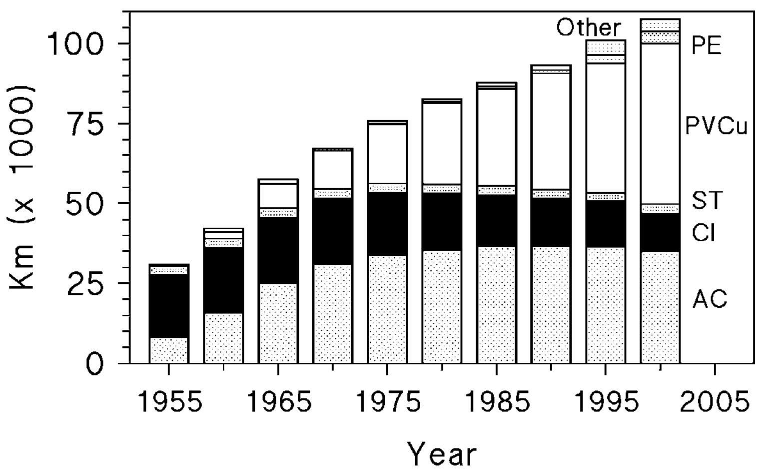

Fig. 1. Pipe materials as used in distribution systems (D>50 mm) in the Netherlands. AC, asbestos cement; CI, cast iron; ST, steel; PVCu, unplasticized PVC; PE, polyethylene; other, e.g. concrete, glass fiber reinforced plastic materials.

Cast iron and asbestos cement were the main pipe materials in the Netherlands in the first half of the 20th century. Presently, unplasticized PVC (PVCu) which as introduced in the fifties of the 20th century has become the main pipe material (50%) in distribution systems (Fig. 1).

Water quality in the distribution system is maintained by prevention of ingress and regrowth, respectively (36). Maintaining a high pressure in the mains is a major measure for ingress prevention, but a series of additional measures which will not be discussed here are needed to ensure a safe distribution of treated water. Regrowth is limited by distributing biologically stable water and the application of biologically stable materials in contact with water. The evaluation of the biological (in)stability of treated water is based on assessment of the AOC concentration and the Biofilm Formation Rate (BFR), respectively (36, 37). The BPP method for determining the growth-promoting properties of materials in contact with water is based on experiences with the AOC and BFR methods, respectively.

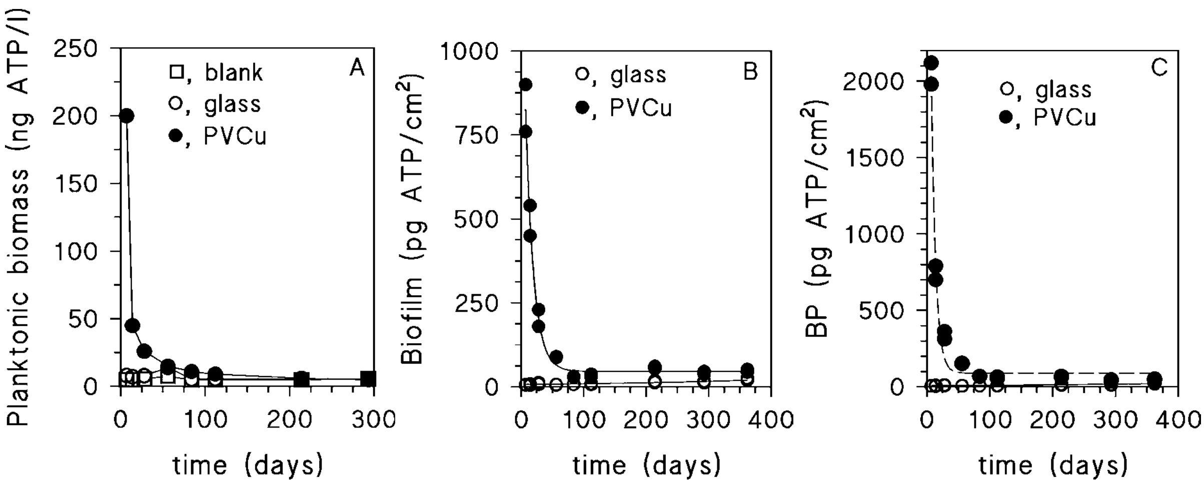

Fig. 1 Biomass production in the presence of unplasticized PVC (PVCu) in the BPP test in tap water at 25°C.

BPP values of PVCu and PE materials

Fig. 2 gives a typical result of the formation of biomass on PVCu in the BPP test. Initially, the concentration of planktonic biomass and biofilm is relatively high and BP_{max} can be calculated from these values. After an exponential decrease stable levels are reached. BP values calculated from the concentrations of planktonic biomass and biofilm, respectively also decreased rapidly and reached a stable level (BPP) of about 60 pg ATP cm^{-2} after about 60 days. This BPP level is a fraction of the value of BP_{max} (about 2100 pg ATP cm^{-2}). The rapid increase followed by a rapid decrease indicates that (i), biodegradable compounds present on the surface of this material were rapidly utilized and (ii), the continuing supply of growth substrates is very limited. For the long term behavior of the material in the distribution system, the latter property is most relevant, but initially high BP values may cause problems directly after installation. The concentration of suspended biomass in the presence of glass remained below 10 ng ATP l^{-1} and the BP values of glass remained below 20 pg ATP cm^{-2} (without correction for the effect of the water).

Examples of biomass production in the presence of PE are given in Fig. 3. The values presented in Fig. 3 are typical examples and depend on material composition and production procedures. These values are not necessarily representative for all PE materials.

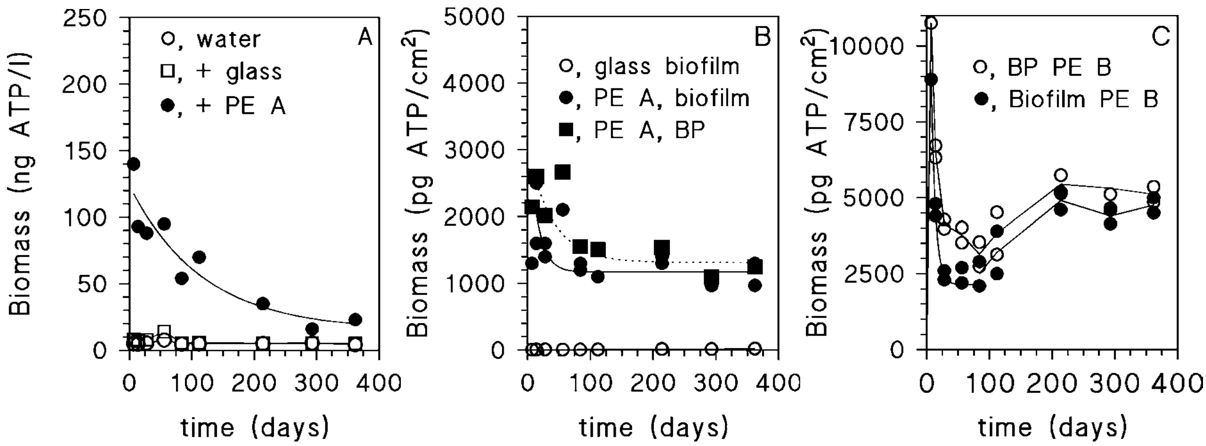

Fig. 3 Biomass production of PE in the BPP test at 25°C. PE A, low density PE, PE B, high density PE.

Biomass production in the presence of PE materials in the BPP test differs from the biomass production with PVCu. The BP_{max} value of PE may be much higher than observed for PVCu and the BPP values remain at a higher level. A typical phenomenon observed with some PE materials is the increase of the BP value after prolonged incubation (> 100 days). Fig. 3c suggests that two different processes occur at the surface of the material. A decreasing concentration of biomass which had been developed on easily available compounds initially present at the surface of the material and a more slowly utilization of compounds coming available after prolonged incubation. The average biomass production rate (BPR) of the slow process can be estimated at about 20 to 25 pg ATP cm^{-2} d^{-1}.

Also plasticized PVC (PVCp) has been tested in the BPP test, serving as a positive control in a number of cases. BPP values of PVCp (11 tests with different materials) ranged from 13400 to 48000 pg ATP cm^{-2}, with a median value of 21800 pg ATP cm^{-2}. PVCp is not used in distribution system and in plumbing systems.

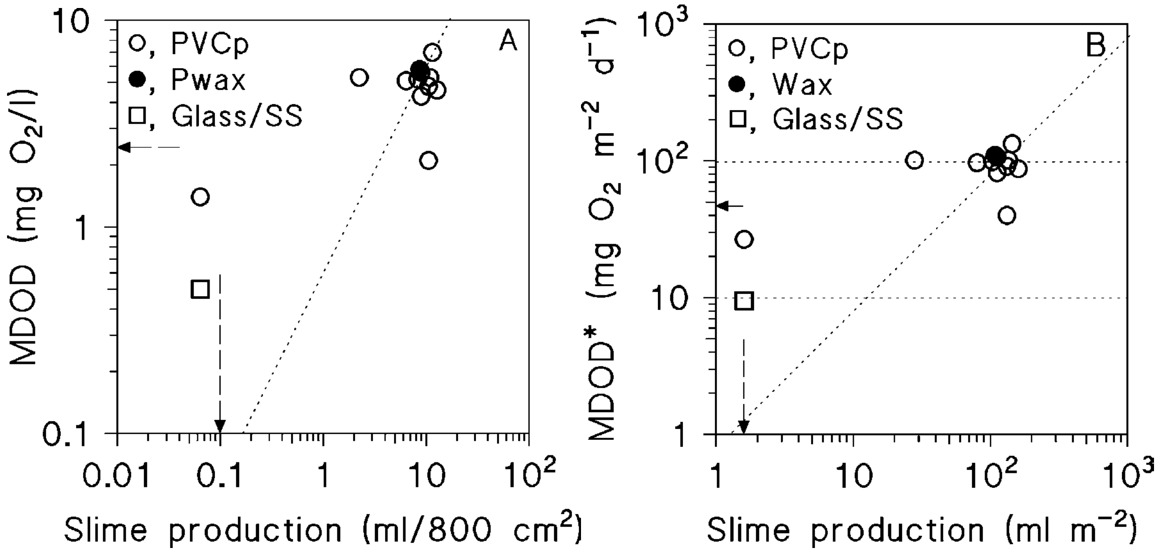

Fig. 4. A comparison of the test results with materials in the MDOD en W270 tests (based on Schoenen and Colbourne, 1978). Pwax: paraffin wax (positive control in MDOD test). Criteria (2.3 mg O_2/l and 0.1 ml slime/800 cm^2) are indicated with arrows. Dotted lines represent proportional relationships. B. MDOD, average oxygen consumption rate as calculated from the MDOD values (see text).*

Criteria

In W270 a criterion of 0.1 ml of slime on 800 cm^2 of material has been defined (31). This value, which is the lower detection level of the method, is equivalent to 1.25 ml of slime/m^2. The MDOD criterion of 2.3 mg O_2 l^{-1} has been derived from observations that visible growth occurs in the test at higher MDOD values (27, 34). In the MDOD test, water is replaced twice a week. An average oxygen consumption rate of 44 mg O_2 $m^{-2}.d^{-1}$ can be derived from an MDOD value of 2.3 mg l^{-1} when it is assumed that the oxygen consumption in the MDOD test is constant over a period of days. Fig. 4 shows the results of a comparison of the MDOD test and the SP test on a number of PVC materials (35). In Fig 4B, MDOD* is expressed as oxygen consumption rate and the SP values are calculated as ml m^{-2}. The growth potential values of the tested PVC materials and the positive control (paraffin wax) were nearly all in the same range, with low values observed with the glass and steel controls. In Fig. 4 lines are drawn which represent a proportional relationship between slime production and MDOD values. These lines are based on the cluster of relatively high values. At low SP values the MDOD values remained relatively high.

An estimation of the effect of a pipe material on the oxygen uptake in treated water can derived from the MDOD value (expressed as oxygen consumption rate, mg O_2 m^{-2} d^{-1}) and the surface to volume ratio (S V^{-1}):

$$\text{Oxygen uptake rate (mg } O_2 \text{ } l^{-1} \text{ } d^{-1}) = S \ V^{-1} \times 0.1 \times MDOD^*$$

where SV^{-1} is the surface to volume ratio in the pipe (cm^{-1}) and MDOD* is the average oxygen consumption rate (mg O_2 m^{-2} d^{-1}) in the MDOD test. In a pipe with an internal diameter of 1cm and a material with an MDOD value of 2.3 mg l^{-1} the oxygen uptake rate is 17.6 mg $l^{-1}.d^{-1}$. With this value oxygen depletion will occur within about 12 hours. Similarly, oxygen depletion would occur after about 4 to 5 days in a pipe with an internal diameter of 10 cm. In small diameter PVCp tubing, with MDOD = 5 mg/l (MDOD*=95 mg O_2 m^{-2} d^{-1}) oxygen depletion will be reached within a few hours. This oxygen depletion may lead to the formation of compounds affecting taste and odor of the water as has been reported (27). These estimations suggest that an MDOD criterion of 2.3 mg/l is relatively high. Furthermore, from the assumption of proportionality between slime production and MDOD it can be derived that the criterion of 0.1 mg l^{-1} for slime production in the SP test (31) is about 35 times more stringent than the criterion for MDOD.

A value of 0.1 ml in the SP test is 100 times lower than the SP values observed for PVCp. BPP values of PVCp range from 13000 to 48000 pg ATP cm^{-2} (see above). Hence, values, which are 100 times below these levels, range from 130 to 480 pg ATP cm^{-2}. The BPP values observed for PVCu are even below this level (< 100 pg ATP cm^{-2}). Distribution of biologically stable drinking water without disinfectant residual in PVCu pipes with these BPP values does not lead to increased HPC values. Consequently, materials with a BPP value <100 ATP cm^{-2} can be used over long distances without causing regrowth problems. Biofilm concentrations on PVCu pipes in distribution systems of unchlorinated supplies in the Netherlands range from less than 100 to more 5000 pg ATP cm^{-2} (36). These biofilm concentrations, which are mainly caused by the uptake of biodegradable compounds from water can be used to define criteria for materials in contact with water (Unifying Biofilm Approach). Establishing such criteria based on BPP values requires further investigations into the relationship between BPP values and regrowth problems, both in distribution systems and in plumbing systems. Such investigations should consider the impact of the SV^{-1} ratio, the length of the pipe (contact time), the presence/absence of disinfectant residual and the multiplication of undesirable bacteria in biofilms. An example is the growth of *Legionella* on rubber materials (18, 19). An objective of these investigations is to describe quality classes for materials, which can be used in defined situations.

Harmonization of test methods

The MDOD, SP and BPP tests have been discussed in CEN Working Groups. Subsequently, legislators in European Community had decided to stimulate harmonization of test methods to achieve a sound basis for the European Acceptance Scheme for materials (39). In a project, which is financially supported by the European Commission, the pipe manufacturers and national governments of participating countries, a study was started in 2001 and results will be reported in 2003. The investigations were focussed on the use of ATP as biomass parameter.

Acknowledgment
The investigations with the BPP test reported in this paper have been conducted as part of the Joint Research Program (BTO) of the water supply companies in the Netherlands.

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