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Managing regrowth in drinking-water distribution systems

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11.1 INTRODUCTION

Colony counts of bacteria on or in solid media containing organic compounds as sources of energy and carbon give information about the concentration of culturable heterotrophic bacteria in water or other environments under investigation. This so-called heterotrophic plate count (HPC), originally developed in 1881 by Robert Koch, was the first tool for monitoring the microbial quality of (treated) water. Within a few years after its introduction, the method was used in many European countries, and soon data came available on HPCs in both raw and treated water (Frankland and Frankland 1894).

A value of 100 cfu/ml had been defined as the first microbial water quality criterion (Koch 1893). However, storage of samples of treated water gave increased plate counts, and it was found that bacteria contributing to HPC values were able to

grow in treated water at very low concentrations of organic compounds (Frankland and Frankland 1894). Many studies were conducted at the end of the 19th century to identify bacteria present in drinking-water and to elucidate their public health significance. For this purpose, pure cultures were inoculated into animals. The results of these studies and the inability of most bacteria to multiply at body temperature demonstrated that HPC values of water had no direct hygienic significance (Zimmermann 1890; Frankland and Frankland 1894; Kayser 1900; Haenle 1903).

At the beginning of the 20th century, the concept of testing water for bacteria of faecal origin was introduced to assess the hygienic safety of treated water (Eijkmann 1904). Methods and media for the detection of faecal indicator bacteria were developed and improved continuously in the course of the century. The Milwaukee outbreak of cryptosporidiosis in 1993 convincingly demonstrated that absence of coliforms does not always ensure microbial safety (Craun *et al.* 1997). Methods for the detection of pathogens are becoming more important for assessing the safety of water treatment. The focus on detection methods for pathogens is the result of both the limitations of the indicator bacteria concept and developments in the field of molecular microbiology, enabling the design of methods for specific and rapid detection of a large variety of bacteria, viruses and protozoa.

Despite the developments in assessing the hygienic safety of treated water, the HPC method has remained a generally applied water quality parameter, and criteria are included in legislation related to the quality of treated water in many countries (e.g., European Union 1998). HPC values provide information about the level of microbial activity in water and therefore can be used to control and optimize treatment processes, procedures and good engineering practices related to water treatment and distribution.

The objective of this chapter is to describe measures for controlling regrowth, with specific emphasis on systems in which treated water is distributed routinely with no or a low disinfectant residual. Brief descriptions are given of the problems caused by biological processes in distribution systems (“regrowth”), methods for determining microbial activity and methods for the assessment of the growth potential (biological stability) of water and materials.

11.2 PROBLEMS RELATED TO MICROBIAL ACTIVITY

11.2.1 Regrowth, biofilms and microbial activity

An increase of HPC values in treated water during distribution is generally described as regrowth or aftergrowth. These descriptions suggest that microorganisms start multiplying in water some time after leaving the treatment facility (Brazos and O’Connor 1996). However, multiplication in distribution

systems mainly takes place on the water-exposed surfaces of the pipes and in sediments, even in the presence of a disinfectant residual. The increase of HPC values in water during distribution thus is mainly due to bacteria originating from biofilms and sediments (LeChevallier *et al.* 1987; van der Wende *et al.* 1989). However, these HPC values are not suited for the identification of water quality problems as may be caused by the multiplication of microorganisms in distribution systems. A number of water quality problems related to microbial activity are described below. More detailed descriptions have been given in earlier reviews (e.g., Olson and Nagy 1984; LeChevallier 1990) and in other chapters in this book.

11.2.2 Coliforms

Multiplication of coliforms in distribution systems has been reported since the beginning of the 20th century. Baylis (1930) found that these organisms grew in sediments accumulating in the distribution system. Howard (1940) also reported multiplication of coliforms in a distribution system during summer. Redwood reservoirs were found to stimulate growth of *Klebsiella* (Seidler *et al.* 1977). Also, coatings were found to stimulate coliform growth (Ellgas and Lee 1980). Wierenga (1985) reported coliform occurrences in distribution systems in the presence of a free chlorine residual. A national survey in the USA revealed that about 18% of the responding companies experienced non-compliance with coliforms, most likely due to multiplication of these organisms in the distribution system (Smith *et al.* 1990). Coliforms can multiply at low substrate concentrations (van der Kooij and Hijnen 1988b; Camper *et al.* 1991). Growth-promoting conditions include concentration of available substrates, water temperature, corrosion, presence of sediments and disinfectant residual (LeChevallier 1990; LeChevallier *et al.* 1996) and are described below and also in chapter 10 of this book.

11.2.3 Opportunistic pathogens

In recent decades, concern has increased about the multiplication of opportunistic pathogens in distribution systems and in plumbing systems. Such organisms include *Aeromonas* spp., *Flavobacterium* spp., *Legionella* species, especially *L. pneumophila*, *Mycobacterium* spp. and *Pseudomonas* spp., especially *P. aeruginosa*. A few characteristics of these organisms are described below. Detailed descriptions of the significance of *Aeromonas* (WHO 2002), *Legionella* (WHO, in revision) and *Mycobacterium* (some non-tuberculous mycobacteria, including *Mycobacterium avium* complex, are the subject of a

separate book in the same series as this volume) in relation to drinking-water safety either have been given elsewhere or are being prepared.

Aeromonas is a common component of the bacterial population of drinking-water in distribution systems but comprises only a small fraction of the heterotrophic population (Leclerc and Buttiaux 1962; Schubert 1976; van der Kooij 1977; LeChevallier *et al.* 1982; Havelaar *et al.* 1990). Reports of Burke *et al.* (1984) caused concern about the possible health effects of *Aeromonas* in drinking-water. In a national survey in the Netherlands, no evidence was obtained that the aeromonads present in drinking-water were enteric pathogens (Havelaar *et al.* 1992). Still, in drinking-water legislation in the Netherlands, a maximum value for *Aeromonas* of 1000 cfu/100 ml is included, aiming at limiting the exposure of the consumer to this organism (VROM 2001).

Pigmented bacteria, including *Flavobacterium* spp., constitute a significant proportion of the HPC values in treated water (Reasoner *et al.* 1989). Certain *Flavobacterium* spp. have been identified as opportunistic pathogens (Herman 1978).

Of the potential pathogens, *Legionella* has attracted most attention, particularly after its discovery in plumbing systems in connection with disease (Tobin *et al.* 1980; Cordes *et al.* 1981; Wadowsky *et al.* 1982). Numerous reports are available about cases of legionellosis caused by exposure to aerosols of warm tap water containing *Legionella*. Certain protozoans grazing on bacteria in biofilms and sediments can serve as hosts for *Legionella* (Rowbotham 1980; Abu Kwaik *et al.* 1998).

Mycobacterium spp., including *M. kansasii*, *M. avium*, *M. chelonae* and *M. fortuitum*, originating from water supplies have been associated with lung infections (McSwiggan and Collins 1974; Engel *et al.* 1980; Kaustova *et al.* 1981; Von Reyn *et al.* 1994). These bacteria, which are highly resistant to chlorine (Carson *et al.* 1978; Haas *et al.* 1983; Taylor *et al.* 2000), can multiply in dead ends of distribution systems and in biofilms (Schulze-Röbbecke and Fischeder 1989; Fischeder *et al.* 1991; Falkinham *et al.* 2001).

P. aeruginosa is not a normal constituent of the bacterial population of treated water (Lantos *et al.* 1969; Hoadley 1977; Hardalo and Edberg 1997), probably because it cannot compete effectively with the related species *P. fluorescens*, which grows at lower temperatures (van der Kooij *et al.* 1982b). Even the fluorescent pseudomonads, which in most cases are unable to multiply at 37 °C, constitute only a small part of the bacterial population of tap water (van der Kooij 1977).

The opportunistic pathogens mentioned above usually remain undetected with the media used for HPC determination, because the organisms either cannot produce colonies on these media or are typically only a very small

fraction of the HPC values. Their detection therefore requires selective media or molecular methods (Manz *et al.* 1993; Schwartz *et al.* 1998).

11.2.4 Increased HPC values

In the second half of the 20th century, granular activated carbon filtration and ozonation were introduced to limit concentrations of undesirable organic compounds in water. These developments and more detailed definitions of microbial water quality criteria increased the focus on HPC values in treated water during distribution. Geldreich *et al.* (1972) concluded that the risk of pathogen contamination increases as the general bacterial population increases and that HPC values (two days, 35 °C) above 500 cfu/ml hampered coliform detection. A problem with HPC values is the diversity of methods used in practice. Typical methods are pour plate count incubated at 35 °C (or 37 °C) for one or two days or at 20–22 °C for two or three days and 20–25 °C spread plate count on diluted agar medium incubated for 7–14 days. Distribution of water treated with ozone as a final treatment step (followed by post-chlorination) gave increased HPC values (three days, 20 °C) ranging from 10^3 – 10^4 cfu/ml in distribution pipes (Berger 1970; Dietlicher 1970; Stalder and Klosterkötter 1976; van der Kooij *et al.* 1977). Incubation of ozonated water in batch tests gave HPC values (three days, 22 °C) above 10^5 cfu/ml, which clearly demonstrated that ozonation increased the growth potential of water (Snoek 1970). In chlorinated supplies, increases of HPC values to more than 10^4 cfu/ml have been reported, usually in situations where chlorine residual became less than 0.1 mg/litre (Rizet *et al.* 1982; Maul *et al.* 1985; Prévost *et al.* 1997, 1998). With the use of R2A medium (seven days, 22 °C), values up to 10^5 cfu/ml were observed (Reasoner and Geldreich 1985; Maki *et al.* 1986). LeChevallier *et al.* (1987) reported HPC values on R2A medium ranging from 320 to 1.3×10^7 cfu/ml in one supply. In a survey in the Netherlands in a summer–autumn period, median HPC values on diluted broth agar medium (14 days, 25 °C) in 19 different water supplies, nearly all without disinfectant residual, ranged from about 150 cfu/ml to 1.6×10^4 /ml. On plate count agar medium (three days, 25 °C), HPC values ranged from 3 to 550 cfu/ml (van der Kooij 1992) (Figure 11.1).

Obviously, some increase of HPC values in treated water during distribution is quite common. The extent of the increase depends on the medium used, the disinfectant residual and the growth-promoting conditions in the systems, as will be discussed below.

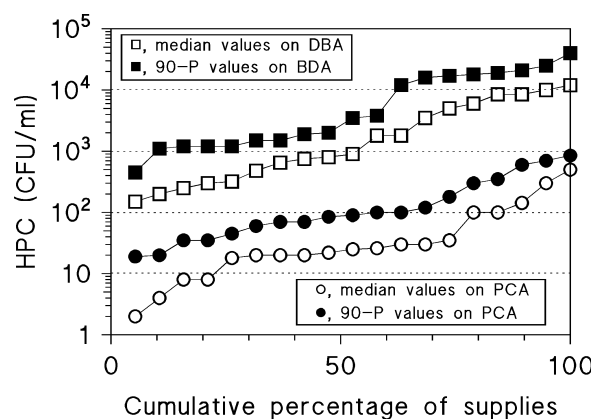


Figure 11.1. HPC values in 19 distribution systems in the Netherlands in a summer–autumn period. PCA, plate count agar, incubated three days at 25 °C; DBA, diluted broth agar, incubated for 10 days at 25 °C. 90-P, 90th-percentile value of the sample series (adapted from van der Kooij 1992).

11.2.5 Nuisance organisms

Discoloured water containing iron bacteria is one of the earliest described problems related to the activity of microorganisms in treated water. De Vries (1890) studied the presence of the iron-precipitating organism *Crenothrix* in the distribution system of the water supply of Rotterdam. This organism had also been observed in other supplies in Europe, even before techniques for culturing bacteria had been developed. Many investigators since then have reported on iron-accumulating bacteria, including *Gallionella* and *Lepthothrix*, in relation to corroding pipes (Clark *et al.* 1967; McMillan and Stout 1977; Tuovinen *et al.* 1980; Ridgway and Olson 1981; Ridgway *et al.* 1981).

Fungi and actinomycetes are usually present in low numbers in distribution systems (Silvey and Roach 1953; Burman 1965; Dott and Waschko-Dransmann 1981; Nagy and Olson 1982, 1985, 1986). These organisms have been associated with taste and odour complaints. Certain actinomycetes are able to degrade natural rubber sealing rings, which may lead to leakage (Leefflang 1968). Water supplies using anaerobic groundwater as a raw water source may contain methane-utilizing bacteria (Schweissfurth and Ruf 1976; Tuschewitski *et al.* 1982). These bacteria do not contribute to HPC values, but their biomass may lead to fouling of the system and serve as food source for protozoa and invertebrates. Also, nitrifying bacteria may be found in such water types when

ammonia removal is incomplete or when monochloramine is used as a disinfectant in distribution systems (Wolfe *et al.* 1990; Skadsen 1993). Growth of these bacteria results in nitrite formation and in an increase in the HPC counts, because components of the biomass of the nitrifying bacteria serve as a food source for heterotrophs. In corroding pipes, sulfate-reducing bacteria are present. These bacteria play a role in microbially induced corrosion, which results in complaints about discoloured water (O'Connor *et al.* 1975; Lee *et al.* 1980; Tuovinen *et al.* 1980; Victoreen 1984; Lee *et al.* 1995). Where bacteria multiply, protozoans may also be present (Michel *et al.* 1995). At elevated temperatures, protozoans with pathogenic properties (*Acanthamoeba*, *Naegleria*) may multiply (de Jonckheere 1979).

The presence of invertebrates in water used for consumption also had attracted attention before bacteriological techniques were used to assess water quality (de Vries 1890). In 1928, Heymann in the Netherlands described the sequence of natural biological processes in distribution systems — namely, multiplication of bacteria, followed by protozoans and subsequently the development of a population of small and larger animals, including *Asellus* (Heymann 1928). Heymann concluded that iron bacteria were a main food source for *Asellus*. In the second half of the century, these animals were studied in a number of other countries (Smalls and Greaves 1968; Levy *et al.* 1986). In the Netherlands, extensive studies have been conducted to obtain information about numbers of invertebrates in unchlorinated water supplies. Asellids comprised the main proportion (>75%) of invertebrate biomass in water flushed from mains, with maximum numbers of *Asellus* ranging from less than 1/m³ to about 1000/m³. Higher maximum numbers (between 10³ and 10⁴/m³) were observed for cladocerans and copepods. For nematodes and oligochaete worms, these levels were usually below 10 and 100 organisms/m³, respectively (van Lieverloo *et al.* 1997).

11.3 ASSESSMENT OF MICROBIAL ACTIVITY

11.3.1 Monitoring tools needed

Biological processes in distribution systems may cause a variety of water quality problems and therefore should be limited. Reliable analytical tools are needed to monitor the extent of the problem, the effects of control measures and the factors promoting microbial activity. Monitoring of HPC values using standard plate count methods is needed because criteria for such HPC values are defined in legislation. However, improved HPC methods and other techniques are

available to elucidate the nature and the extent of the microbial problems and processes.

11.3.2 Heterotrophic plate counts

The HPC value usually represents only a small fraction of the microbial population in water. Major factors affecting the yield of the method include the composition of the medium, the mode of use (spread or pour plate), incubation temperature and incubation time. The medium prescribed for routine monitoring of HPC values contains high concentrations of substrates (beef extract, peptone), and, after a short incubation period (24–48 h), only bacteria growing rapidly on these compounds are enumerated. A large variety of HPC media have been developed since the end of the 19th century, and, in combination with various incubation temperatures and/or incubation periods, different fractions of the community of heterotrophic bacteria can be enumerated. The highest HPC values are obtained with the streak plate method on non-selective media with low substrate concentrations in combination with a long incubation period (Foot and Taylor 1949; Jones 1970; Fiksdal *et al.* 1982; Maki *et al.* 1986). Also, Reasoner and Geldreich (1985) demonstrated the effect of medium composition and incubation time on the HPC yield. The R2A medium, with a relatively low substrate concentration, gave the highest yield after 14 days of incubation at 20 °C. Figure 11.1 shows that diluted broth agar medium gave much higher HPC values than plate count agar medium (van der Kooij 1992). However, even despite these improvements, HPC values on solid media are usually a small fraction (in many cases <1%) of the total bacterial population as enumerated with microscopic techniques (Maki *et al.* 1986; McCoy and Olson 1986; Servais *et al.* 1992). The difference between total direct counts and HPC values is caused by the inability of a majority of bacteria to produce colonies on the applied solid medium, the presence of chemolithotrophic bacteria and the presence of dead cells.

One specific culture medium will never detect all viable heterotrophic bacteria. The best approach for monitoring the multiplication of heterotrophic bacteria in the distribution system is the use of a standardized HPC method with a high yield. Additionally, selective culture methods for the detection of opportunistic pathogens and nuisance organisms can be applied when needed.

11.3.3 Total direct counts

Several techniques are available for enumerating the total number of bacteria in water. The most commonly applied method includes membrane filtration to concentrate bacteria, staining with a fluorescent dye (acridine orange) and

microscopic observation (Hobbie *et al.* 1977). The total direct count (TDC) value obtained in this way is an indicator for bacterial biomass, and observations of specific morphological types of organisms give additional information. Furthermore, the information is available within a short period. TDC values between 10^4 and 10^5 cells/ml have been observed in the distribution systems of Paris (Servais *et al.* 1992) and Metz (Matthieu *et al.* 1995). Prévost *et al.* (1997, 1998) reported values above 10^5 cells/ml for treated water in two Canadian distribution systems and in water from services lines. Concentrations of about 10^6 cells/ml were observed in treated surface water entering a distribution system (Brazos and O'Conner 1996). TDC methods give information about the concentration of cells, but not about the concentration of active biomass, because not all detected organisms are active and because cells have large differences in size. Special methods are available for directly determining the number of viable cells (Coallier *et al.* 1994; McFeters *et al.* 1999).

11.3.4 Adenosine triphosphate

For determining the concentration of active microorganisms, the adenosine triphosphate (ATP) assay has been developed. ATP is an energy-rich compound present in active biomass. The first applications of the ATP analysis for determining microbial activity in water were described by Holm-Hansen and Booth (1966). Values of 250–300 have been reported for the ratio between concentrations of biomass estimated as particulate organic carbon and ATP (Karl 1980). Attractive properties of this analytical method include the following:

- rapidity: the analysis can be conducted within a few minutes;
- low detection level: a concentration of 1 ng ATP/litre can be detected without concentration techniques;
- inclusion of all types of active (micro)organisms;
- ease of interpretation, because ATP concentration is directly related to activity;
- automation: enables the analysis of large series of samples; and
- on-site analysis, using portable equipment.

Improvements of the chemicals and equipment will lead to further decreases in detection limits and improve ease of operation.

ATP analysis is used as a research tool for assessing the presence of microorganisms in drinking-water. In a study conducted in 19 water supplies in the Netherlands, it was found that ATP concentrations in treated water collected

from the distribution systems (mostly without chlorine residual) were usually below 10 ng/litre (Figure 11.2). The HPC/ATP ratio in groundwater supplies ($10^5 - 3 \times 10^5$ cfu/ng) was lower than in surface water supplies ($10^6 - 3 \times 10^6$ cfu/ng), probably because of the presence of nitrifying bacteria coming from filter beds used in groundwater treatment (van der Kooij 1992). Deininger and Lee (2001) observed a high correlation between ATP concentrations and HPC values in 120 samples collected from various systems in the USA. Relatively high ATP concentrations (up to 50 ng/litre) have been reported for a distribution system receiving ozonated water (Bourbigot *et al.* 1982). A survey of all supplies in the Netherlands showed that ATP concentrations in water leaving the treatment plant were below 1 ng/litre in 15% of the samples, with 2.5 ng/litre and 8 ng/litre as median value and 90th-percentile value, respectively (Figure 11.3). Hence, a database for this parameter in treated water is available for reference.

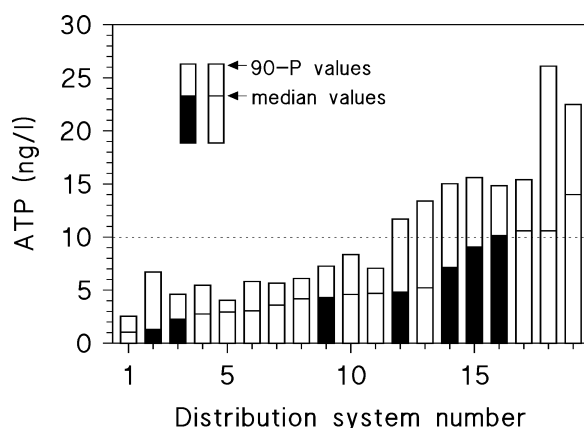


Figure 11.2. ATP concentrations in drinking-water during distribution in 19 water supplies in the Netherlands sampled during summer–autumn. Open bars (bottom) indicate groundwater supplies, black bars indicate surface water supplies. Nos. 2, 3 and 12 have slow sand filtrate as final treatment (adapted from van der Kooij 1992).

11.3.5 Other methods

For determining microbial activity, a large number of enzymatic methods and methods based on the incorporation of radioactive compounds in biomass are available, but these techniques will not be reviewed here. A new and very promising development is the use of molecular methods based on polymerase chain reaction (PCR) or fluorescence *in situ* hybridization (FISH) methods

(Manz *et al.* 1993; Schwartz *et al.* 1998). Such techniques are especially useful in determining the concentrations of specific bacteria that are difficult to culture — e.g., nitrifying bacteria and sulfate-reducing bacteria — but also other types of microorganisms. Developments in this area are fast, and it is expected that rapid molecular techniques will be available in the near future for the quantitative detection of many types of organisms (see chapter 9 of this book).

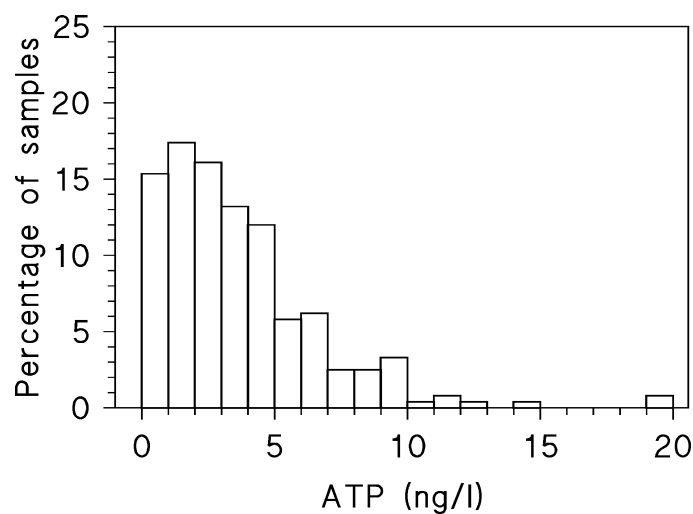


Figure 11.3. Frequency distribution of ATP concentrations in treated water of 243 treatment facilities in the Netherlands. ATP concentrations were above 20 ng/litre in five locations, with a maximum value of 46 ng/litre (unpublished data).

11.3.6 Suite of methods

Monitoring or elucidating microbial activity in distribution systems is best done by using a suite of parameters, namely:

- HPC on a nutrient-poor solid medium, e.g., R2A agar, incubated at 20–25 °C for 7–14 days;
- selective media for detection and isolation of undesirable bacteria, such as *Aeromonas*, *Legionella*, *Mycobacterium*, *Pseudomonas*, etc., when needed;
- ATP, for rapid determination of the total microbial activity; and

- molecular methods (PCR, FISH) for the selective detection of specific microorganisms.

ATP analysis and molecular methods enable a rapid assessment of the microbial water quality, but general application requires further investigations to obtain standardized methods and well defined criteria.

11.4 FACTORS PROMOTING MICROBIAL ACTIVITY

11.4.1 Energy sources in water

11.4.1.1 Power of multiplication

Microbial activity depends on the availability of sources of energy and carbon for formation and maintenance of biomass. The major energy source in treated water is organic carbon, but ammonia may also be present in certain water types. Already in 1885, it was observed that bacteria multiplied in treated water when samples were not processed immediately (Frankland and Frankland 1894). In the early days of microbiology, these observations on the “power of multiplication” of microorganisms caused much excitement, and investigations were conducted for explanation. It was found that even water with a high purity promoted growth of bacteria when stored in a bottle. This power of multiplication was also demonstrated with pure cultures. Attempts were made to quantify the growth potential of water for microorganisms, but in many cases these tests were hampered by the use of flasks plugged with cotton wool, allowing the diffusion of growth-promoting volatile compounds into the test water. Beijerinck (1891) suggested that growth tests with pure cultures of microorganisms should be conducted in boiled water, but results of such tests do not seem available. Heymann (1928) published a method for determining the concentration of assimilable organic compounds in water based on the reduction of the potassium permanganate value after one or more passages of water through a sand filter at a rate of 1 cm/h. With this method, he observed potassium permanganate reductions in raw water above 50% and 20–40% in treated (rapid sand filtration followed by slow sand filtration) water and in groundwater, respectively. These values demonstrate that even after extended treatment or after soil passage, a substantial part of the organic compounds in water remains available to microbial activity, provided that enough contact time is given.

In the 1970s, the increased interest in microbial water quality and the introduction of new treatment methods strengthened the focus on the assessment of the microbial growth potential of treated water. A number of methods have been developed in European countries and in the USA (Table 11.1).

Table 11.1. Methods for determining the microbial growth potential or the concentration of biodegradable organic compounds in treated water

Method (units) ¹	Key parameter	Mode	References
AOC (μg carbon/litre)	Biomass (cfu)	Batch	van der Kooij <i>et al.</i> 1982c; van der Kooij 1992; Kaplan <i>et al.</i> 1993; LeChevallier <i>et al.</i> 1993b
BDOC (mg carbon/litre)	DOC	Batch	Joret and Levi 1986; Servais <i>et al.</i> 1987
BDOC (mg carbon/litre)	DOC	Flow-through	Lucena <i>et al.</i> 1990; Ribas <i>et al.</i> 1991; Kaplan and Newbold 1995
BFR (pg ATP/ cm^2 per day)	Biomass (ATP)	Flow-through	van der Kooij <i>et al.</i> 1995b

¹ BDOC, biodegradable dissolved organic carbon; AOC, assimilable organic carbon; BFR, biofilm formation rate (expressed as amount of ATP per cm^2 of exposed surface and per day); DOC, dissolved organic carbon.

11.4.1.2 Assimilable organic carbon

Assessment of the assimilable organic carbon (AOC) concentration is based on growth measurements with a mixture of two selected pure cultures in a sample of pasteurized water contained in a thoroughly cleaned glass-stoppered Erlenmeyer flask (van der Kooij *et al.* 1982c). The strains used in the AOC test are *Pseudomonas fluorescens* strain P17, which is capable of utilizing a wide range of low-molecular-weight compounds at very low concentrations (van der Kooij *et al.* 1982a), and a *Spirillum* sp. strain NOX, which utilizes only carboxylic acids (van der Kooij and Hijnen 1984). The AOC concentration is calculated from the maximum colony counts of these strains, using their yield values for acetate. Consequently, AOC concentrations are expressed as acetate-carbon equivalents/litre. AOC concentrations in treated water in the Netherlands usually are below $10 \mu\text{g}$ carbon/litre, but values up to about $60 \mu\text{g}$ carbon/litre have been observed in surface water supplies with ozonation included in water treatment (van der Kooij *et al.* 1989; van der Kooij 1992). In all types of treated water, the fraction available to strain NOX was the largest proportion of the AOC concentration, indicating that carboxylic acids were the predominating growth substrates. The AOC concentration utilized by strain P17 was less than $1 \mu\text{g}$ carbon/litre in most types of treated water. With this technique, effects of water treatment and distribution have been determined (van der Kooij 1984, 1987, 1992). The method is used in other countries, usually after modification (Kaplan *et al.* 1993; LeChevallier *et al.* 1993b; Miettinen *et al.* 1999). Table

11.2 shows that AOC values reported for treated water in the USA and in Finland ranged from about 20 to more than 400 µg carbon/litre, with median values of about 100 µg carbon/litre (Kaplan *et al.* 1994; Miettinen *et al.* 1999; Volk and LeChevallier 2000). These values are much higher than those observed in the Netherlands.

Table 11.2. Ranges of concentrations of DOC, BDOC and AOC in treated water as observed in a few surveys (mean values are given in parentheses)

Country	Systems	DOC (mg carbon/litre)	BDOC (mg carbon/litre)	AOC (µg carbon/litre) ¹	Reference
Netherlands	20	0.3–8.6 (3.3)	ND ²	1.1–57 (8.1)	van der Kooij 1992
USA	79	0.2–4.3 (2.0)	0.01–0.97 (0.24)	18–322 (110)	Kaplan <i>et al.</i> 1994
Finland	24	0.6–5.0 (2.7)	ND	45–315 (130)	Miettinen <i>et al.</i> 1999
USA	31 (95) ³	0.6–4.5 (2.0)	0.03–1.03 (0.32)	14–491 (94)	Volk and LeChevallier 2000

¹ Acetate-carbon equivalents/litre.

² ND, not determined.

³ Number of plants for which AOC tests were conducted in parentheses.

11.4.1.3 Biodegradable dissolved organic carbon

The biodegradable dissolved organic carbon (BDOC) method, as developed by Joret and Levi (1986), determines the decrease of the dissolved organic carbon (DOC) concentration in water samples incubated for several days with sand from a biological filter. The BDOC method developed by Servais *et al.* (1987) determines the DOC decrease in water as caused by the indigenous microbial community after an incubation period of 30 days. Table 11.2 shows that typical BDOC values in treated water in the USA range from less than 0.1 mg/litre to about 1 mg/litre, with median values of 0.24–0.32 mg/litre (Kaplan *et al.* 1994; Volk and LeChevallier 2000). These values were clearly higher than the AOC values reported for the same water types. The difference between BDOC and AOC values is caused by using a relatively high concentration of biomass of an adapted microbial community in the BDOC test, whereas low numbers of two pure cultures are used in the AOC test. A rapid assessment of the BDOC value can be obtained from changes in DOC concentrations following the passage of water through a column containing a support with an adapted microbial community (Lucena *et al.* 1990; Ribas *et al.* 1991).

11.4.1.4 Biofilm formation rate

The biofilm formation rate (BFR) value is determined with the use of a biofilm monitor, consisting of a vertical glass column containing glass cylinders (with an external surface of about 17 cm²) on top of each other. This column is supplied with the water to be investigated at a flow of 0.2 m/s (empty column). Cylinders are sampled periodically from the column, and the biomass concentrations of the glass surface are determined with ATP analysis. Subsequently, BFR values are calculated from the biomass increase in time and expressed as pg ATP/cm² per day (van der Kooij *et al.* 1995b). BFR values of treated water in the Netherlands typically range from less than 1 pg ATP/cm² per day in slow sand filtrate to values between 30 and 50 pg ATP/cm² per day in drinking-water prepared from anaerobic groundwater. The system has been calibrated with acetate added to treated water. A concentration of 10 µg acetate-carbon/litre gave a BFR value of 360 pg ATP/cm² per day, and a BFR value of 35 pg ATP/cm² per day corresponds with 1 µg carbon/litre of easily available carbon compounds (van der Kooij *et al.* 1995a).

These observations demonstrate that very low concentrations of readily biodegradable compounds may affect biofilm formation. From the observed BFR values, it can be derived that the concentration of such compounds is less than 1 µg carbon/litre in most supplies with AOC concentrations below 10 µg carbon/litre. Combining the results of the AOC test and the BFR values gives a two-dimensional approach for evaluating the biological (in)stability of treated water.

11.4.2 Materials and sediments

11.4.2.1 Materials

Many reports are available about microbial growth promotion induced by materials in contact with treated water. Such materials included coatings, rubbers and pipe materials (Speh *et al.* 1976; Colbourne and Brown 1979; Ellgas and Lee 1980; Schoenen and Schöler 1983; Frensch *et al.* 1987; Bernhardt and Liesen 1988) and also wood used in service reservoirs (Seidler *et al.* 1977). Certain chemicals used in water treatment — e.g., coagulant or filtration aids — and lubricants can also enhance microbial growth (van der Kooij and Hijnen 1985; White and LeChevallier 1993). A number of materials in contact with treated water can promote the growth of opportunistic pathogens — e.g., *Legionella* and *Mycobacterium* (Colbourne *et al.* 1984; Nideveld *et al.* 1986; Rogers *et al.* 1994; Schulze-Röbbecke and Fischeder 1989). In the United Kingdom, materials in contact with treated water are tested in the mean dissolved oxygen difference (MDOD) test (Colbourne and Brown 1979).

Materials with an MDOD level above 2.3 mg/litre are considered unsuitable for use in contact with treated water (Colbourne 1985). In Germany, a test method based on determining the amount of slime on the surface of a material is applied (Schoenen and Schöler 1983; DVGW 1990). In the Netherlands, the biomass production potential (BPP) test has been developed, which is based on the biofilm formation potential test by including the amount of suspended biomass in the measurements (van der Kooij and Veenendaal 2001). The BPP value (pg ATP/cm²) is defined as the average value of the sum of the concentrations of attached biomass and suspended biomass estimated after 8, 12 and 16 weeks of exposure. Typical BPP values range from less than about 100 pg ATP/cm² for unplasticized polyvinyl chloride to values above 10 000 pg ATP/cm² for certain plastic materials and rubber components (van der Kooij *et al.* 1999).

Reactive metal surfaces — i.e., corroding cast iron — also enhance microbial growth (LeChevallier *et al.* 1993a; Camper *et al.* 1996; Kerr *et al.* 1999), probably by adsorption of organic compounds on iron oxides (Camper *et al.* 1999).

11.4.2.2 Sediments and corrosion products

Sediments accumulating in distribution systems can serve as a food source for bacteria (Baylis 1930; Allen and Geldreich 1978; Allen *et al.* 1980; Martin *et al.* 1982). Detritus originating from biofilm sloughing may contribute to sediment accumulation, but particles present in treated water (e.g., algal cells) and corrosion products have also been observed in sediments (Ridgway and Olson 1981; Brazos and O'Connor 1996). In cast iron pipes, it is difficult to differentiate between sediments and corrosion products. Sediments and corrosion products protect microorganisms from the disinfectant (LeChevallier *et al.* 1990).

11.4.3 Temperature and hydraulic conditions

Water temperature, flow velocity (variations) and residence time have an impact on microbial activity. Biological activity increases about 100% when temperature increases by 10 °C. A temperature of 15 °C has been reported as critical for coliform growth (LeChevallier *et al.* 1996). Flow velocity (changes) affects supply of substrates and disinfectant, biofilm sloughing and sediment accumulation. An increasing residence time in chlorinated supplies results in a decreasing free chlorine concentration (Lu *et al.* 1995; Vasconcelos *et al.* 1997; Prévost *et al.* 1998). Locations with long residence time — e.g., peripheral parts of the distribution system and service reservoirs (Speh *et al.* 1976; LeChevallier *et al.* 1987; Prévost *et al.* 1997) — are vulnerable for regrowth because of

decreased disinfectant residual, the transportation of sediments and increase of water temperature in summer.

11.4.4 Models

A number of models have been developed to describe the relationships between water quality parameters, distribution system conditions and the extent of regrowth. Lu *et al.* (1995) described a mathematical model for transport of substrates and microorganisms in water pipes. The disinfectant consumption rate at the pipe wall plays a significant role in this model, and the chemical oxygen demand is used as the growth substrate parameter. Servais *et al.* (1995) developed the Sancho model for describing BDOC and biomass fluctuations in distribution systems. In this model, BDOC is divided into a fraction that is rapidly utilized and a fraction of complex substrates that are available only after enzymatic activity (hydrolysis). The model has been validated in practice in distribution systems. A third model, developed by Dukan *et al.* (1996), combines a hydraulic model (Piccolo) with a water quality model, including BDOC, chlorine residual and bacteria. From this model, a BDOC value of 0.25 mg/litre and a temperature of 16 °C were derived as threshold values above which problems can be expected. These values are in agreement with observations in practice. It is not clear to what extent these models are predicting the quality changes in distribution systems, because many studies have shown that microbial activity depends on many variables (LeChevallier *et al.* 1996; van der Kooij 1999). The development and improvement of models are continuing (Huck and Gagnon 2002), with the aim to obtain a tool supporting optimal design and water quality management in distribution systems.

11.4.5 Biological stability

Biologically stable water does not promote the growth of microorganisms during its distribution due to a lack of growth substrates (Rittmann and Snoeyink 1984). Defining biological stability in terms of water quality parameters, however, is rather complicated, because microbial activities as described above are affected by a number of different conditions and the properties of the microorganisms. A low concentration of growth-promoting compounds in treated water is an important factor. Many types of heterotrophic bacteria are adapted to aquatic environments with very low concentrations of easily biodegradable compounds, such as amino acids, carboxylic acids and carbohydrates (van der Kooij and Hijnen 1981, 1984, 1985; van der Kooij *et al.*

1982a). Also, undesirable bacteria, such as *P. aeruginosa*, *Aeromonas* spp. and coliforms, multiply rapidly at substrate concentrations of a few micrograms per litre (van der Kooij *et al.* 1982b; van der Kooij and Hijnen 1988a, 1988b; Camper *et al.* 1991). Consequently, the concentrations of such compounds must be very low in treated water. Based on these findings and observations on the effect of water distribution on AOC concentrations, an AOC concentration of 10 µg carbon/litre has been derived as a reference value for biological stability (van der Kooij 1984, 1992; van der Kooij *et al.* 1989). AOC concentrations below this concentration hardly decrease during distribution in unchlorinated supplies, and HPC values (two days, 22 °C) remain below 100 cfu/ml (Schellart 1986; van der Kooij 1992). From studies in the USA, it was concluded that coliform regrowth was significantly reduced in chlorinated supplies at AOC values below 50–100 µg carbon/litre (LeChevallier *et al.* 1991, 1996). Observations on changes of BDOC concentrations in the distribution system of Paris led to the conclusion that treated water with a BDOC value below 0.2 mg/litre has a high degree of biological stability (Servais *et al.* 1992; Dukan *et al.* 1996).

In groundwater supplies in the Netherlands, multiplication of *Aeromonas* was observed at AOC concentrations below 10 µg carbon/litre and HPC values (three days, 22 °C) remaining below 100 cfu/ml. These observations demonstrated the complexity of defining the biological stability of water. For these water types, a clear relationship was observed between the BFR value and the 90th-percentile values of *Aeromonas* concentrations (cfu/100 ml). The risk of exceeding a 90th-percentile value of 200 cfu/ml was less than 20% at BFR values below 10 pg ATP/cm² per day (van der Kooij *et al.* 1999). Consequently, biological stability assessment in the Netherlands is based on determining the AOC concentration as a measure for the concentration of potentially available compounds, and the BFR value is an indication of the rate at which these compounds (and possibly also compounds not included in the AOC test) can cause biofilm accumulation. Still, this combination of parameters does not completely describe biological (in)stability in distribution systems because of the effects of materials, corrosion processes and sediment accumulation.

In some situations, at relatively high concentrations of humic compounds, the availability of phosphorus was found to be growth limiting instead of the energy source. A sensitive method has been developed to assess the concentration of available phosphorus (Lehtola *et al.* 1999; Miettinen *et al.* 1999).

Testing of materials for biological stability is also needed, and methods are available for this purpose in several European countries (see above). At present, in the framework of developing a European Acceptance Scheme for products in contact with treated water, investigations are conducted to harmonize test methods.

11.4.6 Suite of tools

Microbial activity in distribution systems depends on complex processes. Controlling microbial activity requires knowledge about these processes and tools to elucidate water quality parameters and distribution system conditions. These tools include:

- methods for assessment of the biological stability of treated water;
- methods for assessment of the biological stability of materials in contact with treated water; and
- models for describing the effects of water quality parameters and distribution system conditions on microbial activity.

11.5 CONTROLLING MICROBIAL ACTIVITY

11.5.1 General

Controlling (limiting) microbial activity in distribution systems is needed to prevent water quality deterioration resulting in non-compliance with regulations, consumer complaints, disease or engineering problems. Microbial activity in the distribution system largely depends on the introduction of energy sources. As has been described above, such compounds may originate from treated water and from the materials in contact with treated water. Accumulated sediments also promote growth. The following approaches can be used for controlling (limiting) microbial activity:

- distribution of biologically stable drinking-water in a system with non-reactive, biologically stable materials;
- maintaining a disinfectant residual in the entire distribution system;
- distribution of treated water with a low disinfectant residual and a relatively high level of biological stability; and
- optimization of the distribution system to prevent stagnation and sediment accumulation.

11.5.2 Biological stability

11.5.2.1 Water treatment

Biologically stable water can be achieved by applying an appropriate water treatment, which includes biological processes. In surface water treatment in the Netherlands, one or several of the following biological processes are applied:

storage in open reservoirs, soil/dune passage, granular activated carbon filtration, rapid sand filtration and sand filtration. These processes are used in combination with physical and chemical treatment processes such as coagulation/sedimentation and oxidation/disinfection (ozone, chlorine) to obtain multiple barriers against microorganisms, pollutants and biodegradable compounds (Kruithof 2001). Thus, achieving biological stability in surface water treatment is only one objective, and the design and dimensions of water treatment are to a large extent determined by the microbial safety and the removal of undesirable chemical compounds.

Biological filtration processes are effective in AOC and BDOC removal. Significant reductions up to 80% can be obtained within about 10 min contact time (van der Kooij 1984, 1987; Zhang and Huck 1996; Carlson and Amy 1998). When ozone is applied in water treatment, usually two filtration stages are needed to reduce the AOC concentration to a level of about 10 µg carbon/litre (van der Kooij 1984). This second filtration stage is also important for the removal of biomass and particles (e.g., carbon fines; Morin *et al.* 1996) as produced in the first filtration stage. The presence of chlorine in the influent of filter beds should be prevented, because the disinfectant hampers biological activity. Coagulation/sedimentation processes can also result in a considerable AOC reduction (van der Kooij 1984), but Volk *et al.* (2000) did not observe an AOC reduction despite 30–38% BDOC removal.

Aerobic groundwater abstracted from sandy soils has a high degree of biological stability as the result of extended biological processes in the aquifer. Anaerobic groundwater usually contains higher concentrations of organic compounds as well as ammonia and methane. The AOC concentration of anaerobic groundwater treated with aeration and one or two filtration steps is usually below 10 µg carbon/litre, and the low AOC/DOC ratio (about 1 µg AOC/mg DOC) suggests that organic carbon has a high degree of biostability (van der Kooij 1992). In such supplies, HPC values remain below 100 cfu/ml, but *Aeromonas* regrowth has been observed (Havelaar *et al.* 1990), and relatively high BFR values have been observed in treated water (van der Kooij 1999). Biostability was improved by cleaning (or replacing) filter material and/or intensifying aeration. These measures resulted in better removals of methane and ammonia, but also gave lower concentrations of iron and manganese in the filtrate (Reijnen *et al.* 1993).

A new development in water treatment is the application of membrane processes. In 2000, a surface water treatment plant including ultrafiltration and reverse osmosis was installed in the Netherlands. Treated water had a high degree of biostability (Kruithof 2001). However, the effects of membrane filtration processes are not yet clear. Microbial activity decreased in an experimental pipe loop supplied with nanofiltered water (Sibille *et al.* 1997), but

other reports suggest that nanofiltration removes BDOC but not AOC (Escobar and Randall 1999).

11.5.2.2 Materials

Selection of appropriate materials is important to maintain biostability in drinking-water distribution systems. This requires a systematic approach based on reliable test methods and criteria. Much information is available about effects of materials on microbial growth (Schoenen and Schöler 1983; Colbourne 1985; van der Kooij and Veenendaal 2001).

11.5.3 Disinfection

Maintenance of high pressures in the mains and prevention of cross-connections are crucial measures for ingress prevention. Maintaining a disinfectant residual, aimed at further ensuring the microbiological quality of water in the distribution system by protecting against microbial contamination and preventing regrowth, is common practice in most water supplies in North America and Europe (Trussell 1999). The discovery of trihalomethane (THM) formation by chlorination (Rook 1974) has caused much debate, and in a number of European countries the use of chlorine in water treatment and distribution is restricted as much as possible (van der Kooij *et al.* 1999; Kruithof 2001). In situations where treated water is not stable, adding a disinfectant to treated water is the only option to limit regrowth, but this approach has a number of limitations and drawbacks, which are listed below.

11.5.3.1 Chlorine

Chlorine is an effective disinfectant against viruses and bacteria, but to a lesser extent against protozoa. Payment (1999) demonstrated that disinfectant concentrations as used in distribution systems had only a limited effect on pathogens. Free chlorine concentrations up to 0.3 mg/litre must be maintained to prevent regrowth and formation of biofilms (Geldreich *et al.* 1972; Speh *et al.* 1976). This approach has the following limitations:

- Chlorine is a highly reactive compound, which forms undesirable side products (THMs) for which maximum values are defined in legislation — e.g., 200 µg/litre for chloroform, 100 µg/litre for bromoform and dibromochloromethane, and 60 µg/litre for bromodichloromethane, recommended by WHO (1996); 100 µg/litre in Europe (European Union 1998); and 25 µg/litre in the Netherlands (VROM 2001).

- Low concentrations of chlorine affect the taste and odour of drinking-water, causing consumers to complain or to use alternative sources (Burttschell *et al.* 1959; Bryan *et al.* 1973).
- Chlorination increases the AOC concentration in water, probably by oxidation of large organic molecules (van der Kooij 1984, 1987).
- The chlorine residual rapidly declines in the distribution system. Usually after about a 10-h residence time, the concentration has dropped below 0.1 mg/litre. Pipe material, in particular cast iron, plays an important role in chlorine reduction (Lu *et al.* 1995; Vasconcelos *et al.* 1997; Prévost *et al.* 1998). Chlorine also enhances the corrosion process.
- Low concentrations of chlorine are not effective in biofilms and sediments (LeChevallier *et al.* 1988a, 1988b, 1990; Herson *et al.* 1991), explaining why coliforms may be observed in the presence of a free chlorine residual (Wierenga 1985; LeChevallier *et al.* 1996).
- Certain microorganisms can survive or multiply in the presence of low concentrations of chlorine. As a consequence, chlorination is causing a shift in the microbial community (LeChevallier *et al.* 1980; Ridgway and Olson 1982). Norton and LeChevallier (2000) observed that chlorination caused a shift to Gram-positive bacteria. Gräf and Bauer (1973) isolated a chlorine-resistant *Corynebacterium* from tap water. Also, mycobacteria are relatively resistant to disinfectants (Carson *et al.* 1978; Taylor *et al.* 2000). Nagy and Olson (1982) found more filamentous fungi in chlorinated than in unchlorinated supplies. The hygienic consequences of these shifts are not clear.

These limitations show that chlorine is not the ideal method to limit regrowth in distribution systems. However, the required technology is simple and cheap, and maintaining a chlorine residual throughout the distribution system is an essential safety measure when distribution system integrity cannot be assured.

11.5.3.2 Monochloramine

Monochloramine is used on a large scale for distribution system residual maintenance and has replaced free chlorine residuals in many supplies in the USA and also in a few supplies in Europe. Monochloramine is less reactive than chlorine, and its application has a number of advantages, including less THM production, limited effect on taste and odour, greater stability in the distribution system and relative effectiveness against biofilms (LeChevallier *et al.* 1988b, 1990). Distribution systems receiving water with monochloramine had lower

coliform-positive samples than distribution systems with chlorinated water (Neden *et al.* 1992). LeChevallier *et al.* (1996) demonstrated that coliform counts in distribution systems were 35 times higher in chlorinated than in chloraminated water. A remarkable achievement of using monochloramine is the reduction in cases of legionellosis compared with chlorinated supplies, which has been explained by the effect of monochloramine on biofilms (Kool *et al.* 1999). However, using monochloramine has a number of drawbacks, including formation of nitrite (Wolfe *et al.* 1990; Skadsen 1993) and reaction with elastomers. Furthermore, monochloramine is toxic to humans, which limits its maximum concentration in water, and is also toxic to fish (Bull and Kopfler 1991). Finally, monochloramine is less effective than chlorine against suspended microorganisms, and application may also result in a shift in the microbial community (see above).

The change from chlorine to chloramine in many supplies indicates that monochloramine has certain advantages over chlorine. However, when compared with systems maintaining quality without disinfectant, the use of monochloramine is not attractive.

11.5.4 Distribution system configuration and maintenance

Reduction of microbial activity can also be achieved by measures in the distribution system. Such measures include preventive actions and corrective activities. Improved system design for maintaining water quality during distribution aims at reducing residence time and stagnation and the use of non-corrosive materials. Conditioning of the water to limit corrosion also appears to be effective in regrowth prevention (LeChevallier *et al.* 1993a). Corrective measures such as cleaning by flushing or pigging have only a limited effect, because these techniques are difficult to apply in transmission mains and trunk lines (LeChevallier *et al.* 1987).

11.5.5 Multiple barriers against microbial activity in distribution systems

Microbial activity in the distribution system is affected by many factors. Therefore, controlling microbial activity can be achieved only with a combination of measures (multiple barriers). Removal of biodegradable compounds from the water is of major importance, but a systematic approach in eliminating or preventing growth-promoting conditions in the distribution system is also essential. When biostability is not achieved, maintaining a disinfectant residual is necessary to prevent water quality deterioration. The

level of disinfectant needed to control microbial activity may be related to the degree of instability, but local conditions (water composition, size of distribution system, water temperature) will also have a large impact. Consequently, a tailor-made solution requires a systematic analysis of the potential hazards to define appropriate control measures and critical control points. This approach should be part of a water safety plan that covers all aspects of drinking-water safety.

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