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Biodiversity in drinking water distribution systems: a brief review

J. C. BLOCK[1], I. SIBILLE[2], D. GATEL[3], D. J. REASONER[4], B. LYKINS[4] AND R. M. CLARK[4]

[1]*Nan. CIE/GIP Stelor/LSE, Faculté de Pharmacie, 5 rue Albert Lebrun, 54000 Nancy, France*

[2]*LHRSP, 11 bis rue Gabriel Péri, 54500 Vandoeuvre, France*

[3]*Compagnie Générale des Eaux, Quartier Valmy, 32 Place Ronde, Cedex 82, 92982 Paris La Defense, France*

[4]*USEPA, Office of Research and Development, National Risk Management Research Laboratory, 26 W Martin Luther King Drive, Cincinnati, Ohio 45268, USA*

In drinking water distribution systems, three groups of living organisms are usually found in the biofilm and circulating water: heterotrophic bacteria, free-living protozoa, and macro-invertebrates. Indirect evidence suggests that protozoa grazing in distribution systems can partially eliminate biomass production and accidental microbiological pollution.

Introduction

Drinking water distribution systems are constantly exposed to an inflow of biodegradable organic substances and foreign microorganisms (bacteria, fungi, protozoa, etc.) not only from water treatment plants, but also from reservoirs and through accidents occurring on the distribution networks (burst pipes, repairs and cross-connections). Some of these microorganisms (particularly heterotrophic bacteria) easily adapt to this oligotrophic environment and can colonize the whole system even when chlorine is used as a residual disinfectant. They eventually form a stable ecosystem that is almost impossible to eradicate (Maul *et al.* 1991; Sibille *et al.* 1995). The highest density of microorganisms is found on the surfaces of structural materials in the form of more or less dispersed microcolonies (biofilm) mixed with corrosion products and inorganic precipitates (Block *et al.* 1997). Accumulation at this level is explained by the transport of bacteria and nutrients to the surface and bacterial adhesion (Bryers 1987), the multiplication of attached bacteria, a nutritional advantage for fixed bacteria (compared with waterborne bacteria), and limited diffusion of residual oxidant through the boundary layer at the water/material interface (Fig. 1). In pipes <10 cm diameter, there are on average 50 to 100 times more fixed bacteria than planktonic bacteria (i.e. suspended in water).

The purpose of this short bibliographic review is twofold: on the one hand to briefly recall the biological diversity found in drinking water distribution systems and the trophic interactions between the different species, and on the other hand to analyze the important role played by protozoan grazing in removing a large proportion of the microbial biomass produced in the systems.

Biodiversity and the food chain

In water distribution systems, three groups of living organisms are normally found in the biofilm and circulating water (Table 1): heterotrophic bacteria, free-living protozoa (amoebae, ciliates and flagellates), and macroinvertebrates (rotifers, nematodes and microcrustaceans). A very large number of bacterial species have been identified in distribution systems (see review

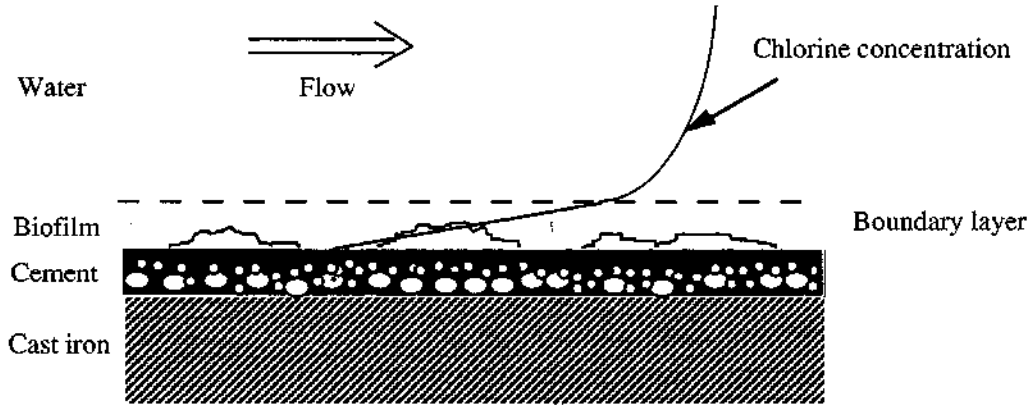

Figure 1. Schematic representation of a longitudinal section through the pipe of a water distribution system, showing biofilm accumulated at the surface of the material within the boundary layer.

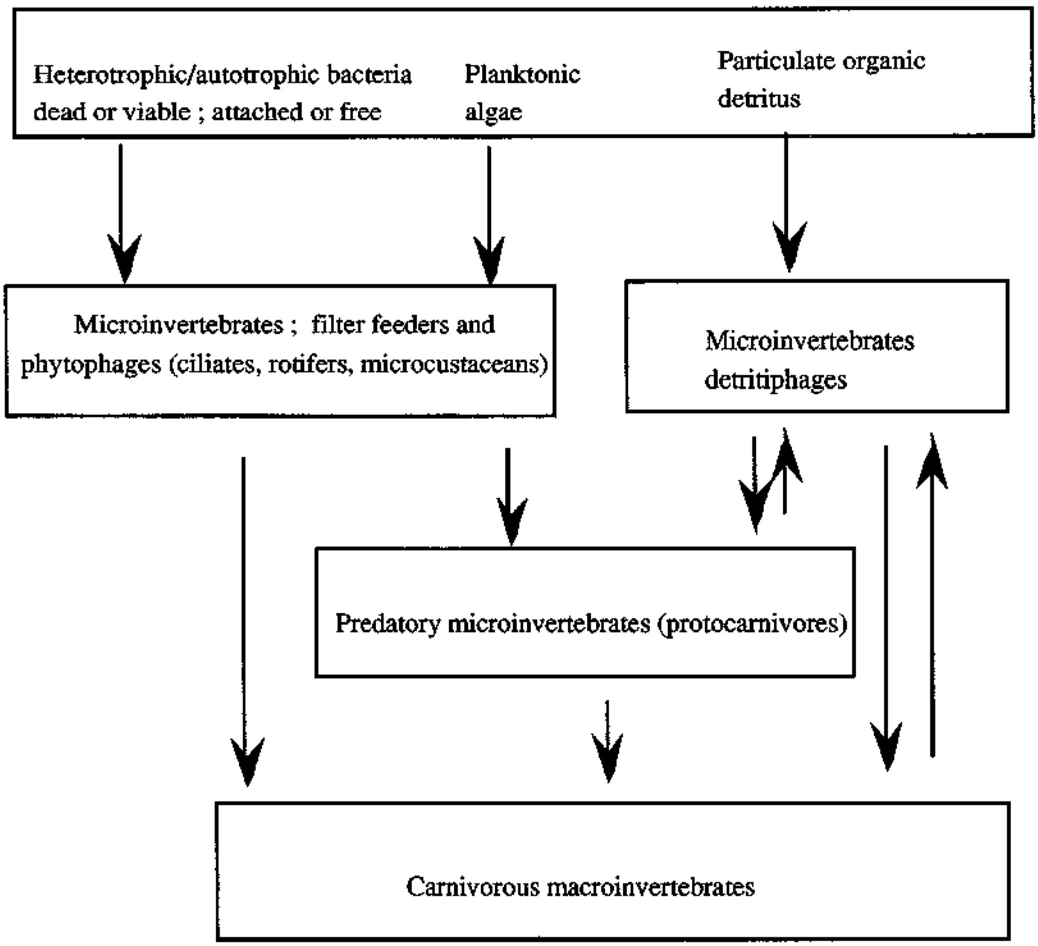

Figure 2. Food chain in a water distribution system (adapted from Mouchet & Pourriot 1992).

by Dott 1983). These bacterial species seem to be as diverse as those found in natural systems, since more microorganisms develop opportunistic strategies for occupying environments with different temperatures, nutrients and toxins.

Bacterial biomass and organic debris represent the start of a complex food chain in which the greatest consumers of aquatic bacteria are the smallest zooplankton members such as microinvertebrate filter-feeders, phytophages and detritiphages (Fig. 2). They are considered to be a key link between bacteria and the other food-chain components (Carrias *et al.* 1994; Fenchel & Harrison 1976; Fenchel 1987; Porter 1984). The diagram in Figure 2 might be somewhat sketchy. For example, Rogers *et al.* (1994) remind us that amoebae consume bacteria but that some of them (*Lacrymaria*) are predators of other protozoa. Moreover, the activity of higher trophic levels has not yet been demonstrated in distribution systems and the issue of the mass balance of organic matter in the systems has not been solved either. Lastly, seasonal variations of the different trophic levels have been reported in drinking water reservoirs (Amblard *et al.* 1996).

Table 1. *Densities of some groups of free-living organisms in distribution systems.* +++ indicates abundant.

Organisms	Densities	Reference
Bacterial cells	Biofilm: 10^5 to 10^8 cm^{-2} Water: 10^3 to 10^7 cm^{-2}	Block *et al.* 1997
Protozoa	0.1 to 365 ml^{-1}	Rogers *et al.* 1994; Laurent 1995; Servais *et al.* 1995; Amblard *et al.* 1996
Macroinvertebrates (nematodes and microcrustaceans)	Biofilm: +++ Water: +++ (*Asellus:* 1000 m^{-3})	Boët 1984; Levy *et al.* 1984

Table 2. *Some examples of identified free-living protozoa in the natural environment and in drinking water distribution systems.*

Systems	Protozoa	Reference
Soils	*Colpoda steinii* (a ciliate)	Wright *et al.* 1995
Ground water	Flagellates	Sinclair *et al.* 1990, 1993
Surface water	*Colpoda inflata, Uronema* sp. (ciliates)	Iriberri *et al.* 1995
Potable water distribution systems	*Hartmannella, Naegleria*	Chang *et al.* 1960
	Acanthamoeba, Hartmanella, Naegleria	Jacquemin *et al.* 1974, 1981; Simitzis-Le Flohic 1976; Simitzis & Jacquemin 1977
	Bodo, Cochliopodium Naegleria, Hartmanella, Vanella	Block *et al.* 1993
	Hartmanella, Lacrymaria spp., *Virillifera*	Rogers *et al.* 1994
	Bodo, Chilodonella, Monas, Monosiga, naked amoebae	Amblard *et al.* 1996
Activated sludge	Ciliated protozoa (up to 30 species)	Al-Shahwani & Horan 1991

Usually, protozoa have been detected and studied in aquatic systems either during episodes of macroscopic pollution by macroorganisms, or systematically in the 1960s and 1970s when the first cases of meningeal encephalitis caused by amoebae in swimming pools were observed. The most commonly encountered genera were *Acanthamoeba, Hartmannella* and *Naegleria* (Table 2). Overall, there has been little systematic, documented research on protozoa in distribution water and biofilms.

Activity of free-living non-pathogenic protozoa in water distribution systems

The activity of free-living protozoa in their natural environment has been extensively studied (see Finlay 1997, this volume). Photographs and movie-film recordings of biofilm on sections of materials immersed in drinking water have made it possible to closely observe protozoa grazing on free-living and/or fixed bacteria (Fig. 3) in distribution systems (Pedersen 1990; Rogers *et al.* 1994), but the activity of protozoa consuming bacteria (clearance = ingestion rate divided by the number of protozoa) under these conditions has never been published. There is no reason to believe, however, that what we know of protozoa in natural waters could not be applied to protozoa in water distribution systems.

Bacterial clearance by protozoa varies greatly, depending on the system studied: for example, the numbers of bacteria cleared hourly per protozoon were 10 to 600 in wastewater and activated sludge (review by Ratsak *et al.* 1996), and 30 to 100 (Menon *et al.* 1994) and 5 to 80 (Iriberri *et al.* 1994) in river water.

This strong activity explains why 80% of foreign bacteria in surface water (Carrias *et al.* 1994; Menon *et al.* 1994), 10 to 90% of river-water bacteria (Iriberri *et al.* 1994, 1995) and 91% of the biomass produced in activated carbon filters from drinking water plants (Servais *et al.* 1991), are removed by grazing. The findings of Iriberri *et al.* (1994) also show that there is an identical amount of grazing for both foreign and native microorganisms in river water, thus indirectly suggesting that protozoa can partially eliminate accidental microbiological pollution in distribution systems.

All of the evidence on distribution systems is indirect. Two examples are given below.

The first example concerns the relationship between the total number of bacteria (expressed as acridine orange direct count, AODC) and protozoa in water from different French distribution systems, published by Servais *et al.* (1995) (Fig. 4). The highest bacterial densities correspond with the largest numbers of protozoa. On the other hand, very few protozoa are found if bacterial concentrations are lower than 10^7 l^{-1}, thereby backing up the notion that for maximum activity, some protozoa need an optimal bacterial density (see review by Ratsak *et al.* 1996).

The second example concerns the ability of an *Escherichia coli* strain to multiply and survive in drinking water distribution systems. We have already shown that a small fraction of an *E. coli* colony seeded in an experimental system can grow and temporarily colonize it (Block *et al.* 1995; Fass *et al.* 1996). The intensity of this colonization varies with the energy resources in the system, which depend on the quality of the water introduced in the system (water coming from a granular activated carbon filter *versus* nanofiltered water).

The characteristics of two systems tested are compared in Table 3. When *E. coli* were seeded in either system, growth was faster and more intense in the system supplied with nanofiltered water (Fig. 5). This difference cannot be explained directly as there were low, non-measurable biodegradable organic matter concentrations, and a native active bacterial flora was always present. The findings of this research were completely unexpected and have been confirmed several times. They could be explained by a lower density of protozoa in the system supplied with nanofiltered water and thus the grazer population would not be able to consume this wave of introduced microorganisms.

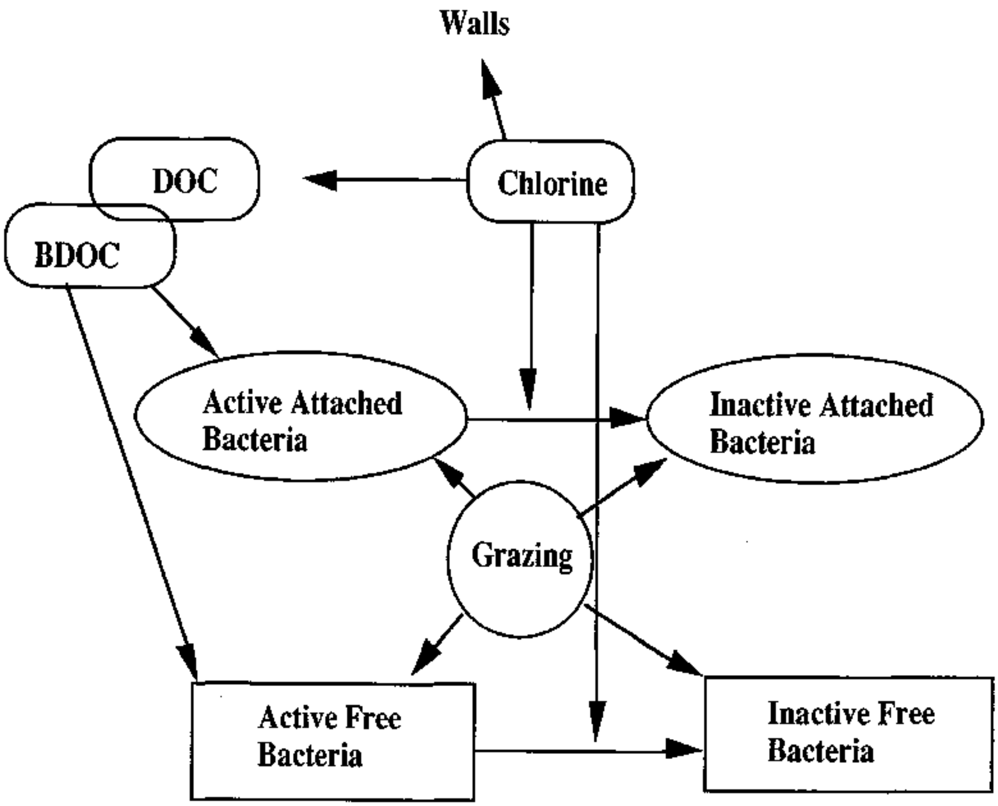

Figure 3. Description of potential interactions between organic matter, bacteria, protozoa and chlorine in main distribution systems.

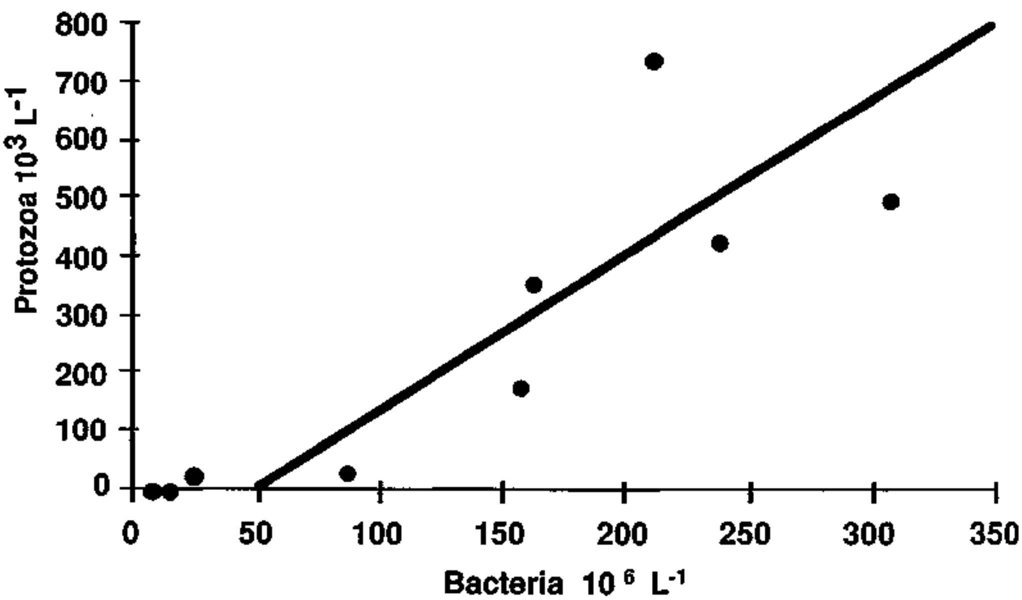

Figure 4. Average abundance of protozoa plotted against average abundance of free bacteria. (From Servais *et al.* 1995, with permission).

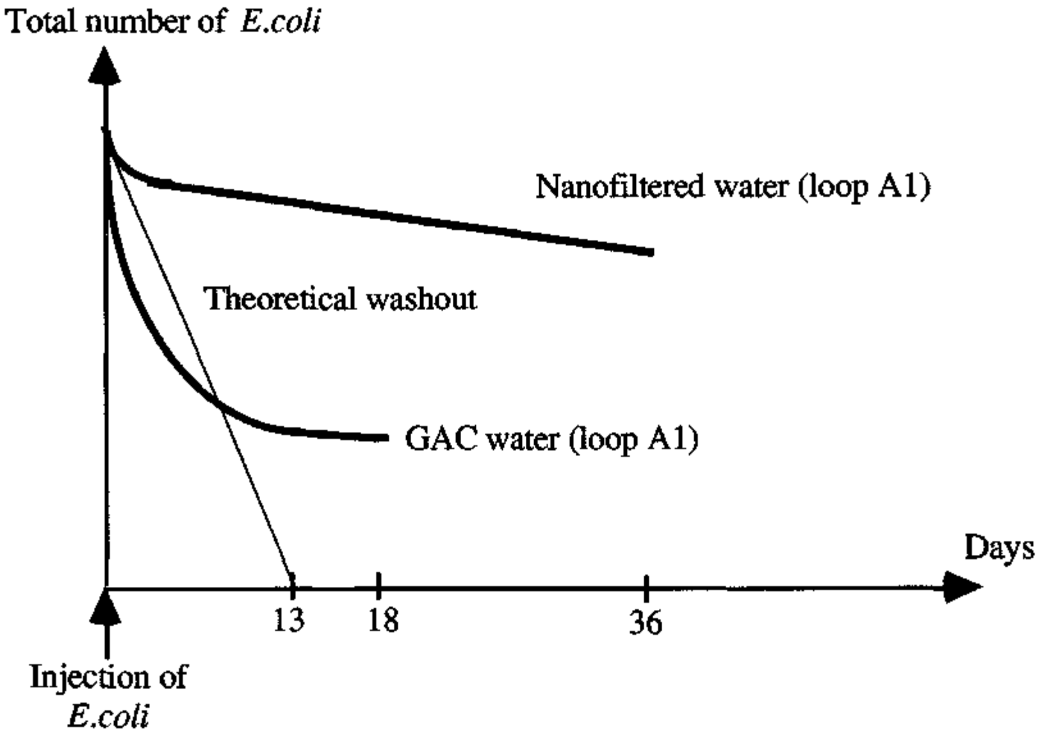

Figure 5. Total counts of *Escherichia coli* in two networks (loops A1) supplied with water filtered through granular activated carbon (GAC) or a nanofilter.

Table 3. *Average characteristics of two networks fed with water filtered through granular activated carbon (GAC) or a nanofilter, at quasi steady-state (hydraulic residence time 12 h; temperature 20°C) before single injections with a suspension of* Escherichia coli.

Network	Total numbers of bacteria (DAPI counting) (no. l^{-1})	(no. cm^{-2})	Chlorine (mg Cl_2 l^{-1})	DOC (mg C l^{-1})	Protozoa
GAC water	6.6×10^5 (1% viable)	4.2×10^6 (3% viable)	0	1.7 (BDOC: 0.4)	?
Nanofiltered water	4.5×10^5 (0.02% viable)	4.6×10^6 (2% viable)	0	0.3 (BDOC: not measureable)	?

Conclusions

The systematic detection of bacterial biomass and protozoa in drinking water distribution systems shows the constant maintenance of a well diversified microbial ecosystem that remains stable even when disinfectants are added, as already observed in swimming pools (Rivera *et al.* 1993) which are generally more heavily chlorinated than drinking water systems.

No research seems to have been done on either the trophic chain or activity of protozoa in drinking water distribution systems. Conversely, free-living protozoa and macroinvertebrates have often been accused of protecting bacteria from the action of disinfectants, by accumulating them in the digestive tract and vacuoles (Levy *et al.* 1984; King *et al.* 1988; Shotts & Wooley

1990; Barker *et al.* 1992). However, it is also important to recognize the potential ability of these organisms to remove a high proportion of the biomass introduced and produced in distribution systems.

Several questions must be answered before we can consider using the biocontrol and biocleaning properties of protozoa for controlling the quantities of bacteria in water distribution systems. How many types of amoebae, ciliates and flagellates occur and what densities of protozoa are found in distribution systems? What is the balance between predators and prey? What are the effects of hydraulic discontinuities and biomass type on the activities of the protozoa? Does chlorination (or use of other disinfectants) of distribution systems partially and temporarily destroy this trophic link?

This short review is based on the work conducted within the framework of the Biofilm research programme (Nan.C.I.E., GIP Stelor, C.G.E., S.E.D.I.F., Anjou-Recherche, Pont-à-Mousson S.A., U.S.E.P.A., O.N.E.P.).

References

Al-Shahwani, S. M. & Horan, N. J. (1991). The use of protozoa to indicate changes in the performance of activated sludge plants. *Water Research*, **25**, 633-638.

Amblard, C., Bourdier, G., Carrias, J. F., Maurin, N. & Quiblier, C. (1996). Seasonal evolution of microbial community structure in a drinking water reservoir. *Water Research*, **30**, 613-624.

Barker, J., Brown, M. R. W., Collier, J. J., Farrel, I. & Gilbert, P. (1992). Relationship between *Legionella pneumophila* and *Acanthamoeba polyphaga*: physiological status and susceptibility to chemical inactivation. *Applied Environmental Microbiology*, **58**, 2420-2425.

Block, J. C., Clark, R. M., Deguin, A., Fontvieille, D. *et al.* (1997). BOM in water distribution systems. In *Biodegradable Organic Matter in Drinking Water* (eds M. Prévost & J. C. Joret), Chapter 4. (Lewis, in press).

Block, J. C., Haudidier, K., Paquin, J. L., Miazga, J. & Lévi, Y. (1993). Biofilm accumulation in drinking water distribution systems. *Biofouling*, **6**, 333-343.

Block J. C., Mouteaux, L., Gatel, D. & Reasoner, D. J. (1995). Survival and growth of *E. coli* in drinking water distribution systems. In *Conference "Coliforms and* E. coli: *problem or solution?", Leeds, UK, September 1995.* (Royal Society of Chemistry).

Boët, P. (1984). The biology of *Asellus aquaticus* (Crustacea, Isopoda) and its destruction means when invading the drinking water distribution systems: a review. *Sciences de l'Eau*, **3**, 93-110.

Bryers, J. D. (1987). Biologically active surfaces: processes governing the formation and persistence of biofilms. *Biotechnological Progress*, **3**, 57-68.

Carrias, J. F., Amblard, C. & Bourdier, G. (1994). Développement des communautés bactériennes pélagiques lacustres et prédation des protozoaires. Colloque *Le Point sur la Microbiologie de l'Environnement.* Institut Pasteur, Paris, 40 pp.

Chang, S. L., Woodward, R. L. & Kabler, P. W. (1960). Survey of free-living nematodes and amoebas in municipal supplies. *Journal of the American Water Works Association*, **52**, 613-618.

Dott, W. (1983). Qualitative und quantitative Bestimmung von Bakterien-populationen aus aquatischen Biotopen 6. Mitteilung: Wiederverkeimung in Trinkwasser. *Zentralblatt Bakteriology Hygien, I Abt. Orig.*, **B, 178**, 263-279.

Fass, S., Dincher, M. L., Reasoner, D. J., Gatel, D. & Block, J. C. (1996). Fate of *Escherichia coli* experimentally injected in a drinking water distribution pilot system. *Water Research*, **30**, 2215-2221.

Fenchel, T. (1987). *Ecology of Protozoa: The Biology of Free-living Phago-trophic Protists.* Brocks/Springer Series in Contemporary Bioscience. Scientific & Technical Publications, Madison, Springer-Verlag, Berlin.

Fenchel, T. & Harrison, P. (1976). The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes* (eds J. M. Anderson & A. MacFadyen), pp. 285-299. Blackwell Scientific Publications, Oxford.

Finlay, B. J. (1997). The diversity and ecological role of protozoa in fresh waters. In *The Microbiological Quality of Water* (ed. D. W. Sutcliffe), pp. 113-125. Freshwater Biological Association, Ambleside.

Iriberri, J., Ayo, B., Artolozaga, I., Barcina, I. & Egea, L. (1994). Grazing of allochthonous *versus* autochthonous bacteria in river water. *Letters in Applied Microbiology*, **18**, 12-14.

Iriberri, J., Ayo, B., Santamaria, E., Barcina, I. & Egea, L. (1995). Influence of bacterial density and water temperature on the grazing activity of two freshwater ciliates. *Freshwater Biology*, **33**, 223-231.

Jacquemin, J. L., Jacquemin, P., Le Flohic, A. M. & Olory-Togbe, P. (1974). Présence d'amibes de type *Limax* dans les eaux de distribution de l'agglomération de Rennes. *Bulletin de la Société Pathologie Exotique*, **67**, 253-260.

Jacquemin, J. L., Simitzis-Le Flohic, A. M. & Chaveau, N. (1981). Recherche des amibes libres dans l'eau. Etude du réseau d'alimentation en eaux de la ville de Poitiers. *Bulletin de la Société Pathologie Exotique*, **74**, 521-524.

King, C. H., Shotts, E. B. Jr., Wooley, R. E. & Porter, K. G. (1988). Survival of coliforms and bacterial pathogens within protozoa during chlorination. *Applied Environmental Microbiology*, **54**, 3023-3033.

Laurent, P. (1995). *Colonisation bactérienne des réseaux de distribution: étude expérimentale et modélisation.* PhD Dissertation, Université Libre de Bruxelles, 185 pp.

Levy, R. V., Cheetham, R. D., Davis, J., Winer, G. & Hart, F. L. (1984). Novel method for studying the public health significance of macroinvertebrates occurring in potable water. *Applied Environmental Microbiology*, **47**, 889-894.

Maul, A., Vagost, D. & Block, J. C. (eds) (1991). *Microbiological Analysis in Water Distribution Networks: Sampling Strategies, Methods and Computer Programs.* Ellis Horwood Publishers, New York, 127 pp.

Menon, P., Servais, P. & Becquevort, S. (1994). Mortalité des bactéries allochtones après rejet en milieu aquatique. Colloque *Le Point sur la Microbiologie de l'Environnement.* Institut Pasteur, Paris, 40 pp.

Mouchet, P. & Pourriot, R. (1992). Pénétration et développement de microinvertébrés dans les réseaux de distribution d'eau potable. *Techniques Sciences Méthodes*, **7-8**, 353-368.

Pedersen, K. (1990). Biofilm development on stainless steel and PVC surfaces in drinking water. *Water Research*, **24**, 239-243.

Porter, K. G. (1984). Natural bacteria as food resources for zooplankton. In *Current Perspectives in Microbial Ecology* (eds M. J. Klug & C. A. Reddy), pp. 340-345. American Society for Microbiology, Washington, DC.

Ratsak, C. H., Maarsen, K. A. & Kooijman, S. A. L. M. (1996). Effects of protozoa on carbon mineralization in activated sludge. *Water Research*, **30**, 1-2.

Rivera, F., Ramirez, E., Bonilla, P., Calderon, A. *et al.* (1993). Pathogenic and free-living amoebae isolated from swimming pools and physiotherapy tubs in Mexico. *Environmental Research*, **62**, 43-52.

Rogers, J., Dausett, A. B., Demis, P. J., Lee, J. V. & Keevil, C. W. (1994). Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophilla* in potable water systems. *Applied Environmental Microbiology*, **60**, 1842-1851.

Servais, P., Billen, G., Ventresque, C. & Bablon, G. P. (1991). Microbial activity in GAC filters at the Choisy-le-Roi treatment plant. *Journal of the American Water Works Association*, **83**, 62-68.

Servais, P., Laurent, P. & Randon, G. (1995). Comparison of the bacterial dynamics in various French distribution systems. *Journal Water SRT-Aqua*, **44**, 10-17.

Shotts, E. B. & Wooley, R. E. (1990). Protozoan sources of spontaneous coliform occurence in chlorinated drinking water. In *Project Summary, Research and Development.* EPA/600/S2-89/019, Cincinnati, Ohio, USA.

Sibille, I., Mathieu, L., Paquin, J. L., Hartemann, Ph. *et al.* (1995). Improvement of water quality during distribution using nanofiltration process. In *American Water Works Association "Water Quality Technology Conference", Louisianna, U.S.A. (November 1995), Part II*, pp. 1383-1390. American Water Works Association.

Simitzis, M. R. & Jacquemin, J. L. (1977). Présence d'ambies libres de type *Limax* dans des eaux de agglomération urbaine de Rennes et de Liffré. *Bulletin de la Société Pathologie Exotique*, **70**, 217-221.

Simitzis-Le Flohic, A. M. (1976). Présence d'ambies libres dans les réseaux d'alimentation urbaine et communale de la région brestoise. *Bulletin de la Société Pathologie Exotique*, **69**, 302-309.

Sinclair, J. L., Kampbell, D. H., Cook, M. L. & Wilson, J. T. (1993). Protozoa in subsurface sediments from sites contaminated with aviation gasoline or jet fuel. *Applied Environmental Microbiology*, **59**, 467-472.

Sinclair, J. L., Randtke, S. J., Denno, J. E., Hathaway, L. R. & Ghiorse, W. C. (1990). Survey of microbial populations in burried-valley aquifer sediments from northeastern Kansas. *Ground Water*, **28**, 369-377.

Wright, D. A., Killham, K., Glover, L. A. & Prosser, J. I. (1995). Role of pore size location in determining bacterial activity during predation by protozoa in soil. *Applied Environmental Microbiology*, **61**, 3537-3543.