APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1998, p. 197–202 Vol. 64, No. 1
0099-2240/98/$04.00+0

Protozoan Bacterivory and *Escherichia coli* Survival in Drinking Water Distribution Systems

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Received 17 June 1997/Accepted 28 October 1997

The development of bacterial communities in drinking water distribution systems leads to a food chain which supports the growth of macroorganisms incompatible with water quality requirements and esthetics. Nevertheless, very few studies have examined the microbial communities in drinking water distribution systems and their trophic relationships. This study was done to quantify the microbial communities (especially bacteria and protozoa) and obtain direct and indirect proof of protozoan feeding on bacteria in two distribution networks, one of GAC water (i.e., water filtered on granular activated carbon) and the other of nanofiltered water. The nanofiltered water-supplied network contained no organisms larger than bacteria, either in the water phase (on average, 5×10^7 bacterial cells liter^{-1}) or in the biofilm (on average, 7×10^6 bacterial cells cm^{-2}). No protozoa were detected in the whole nanofiltered water-supplied network (water plus biofilm). In contrast, the GAC water-supplied network contained bacteria (on average, 3×10^8 cells liter^{-1} in water and 4×10^7 cells cm^{-2} in biofilm) and protozoa (on average, 10^5 cells liter^{-1} in water and 10^3 cells cm^{-2} in biofilm). The water contained mostly flagellates (93%), ciliates (1.8%), thecamoebae (1.6%), and naked amoebae (1.1%). The biofilm had only ciliates (52%) and thecamoebae (48%). Only the ciliates at the solid-liquid interface of the GAC water-supplied network had a measurable grazing activity in laboratory test (estimated at 2 bacteria per ciliate per h). Protozoan ingestion of bacteria was indirectly shown by adding *Escherichia coli* to the experimental distribution systems. Unexpectedly, *E. coli* was lost from the GAC water-supplied network more rapidly than from the nanofiltered water-supplied network, perhaps because of the grazing activity of protozoa in GAC water but not in nanofiltered water. Thus, the GAC water-supplied network contained a functional ecosystem with well-established and structured microbial communities, while the nanofiltered water-supplied system did not. The presence of protozoa in drinking water distribution systems must not be neglected because these populations may regulate the autochthonous and allochthonous bacterial populations.

Drinking water distribution systems are colonized by saprophytic heterotrophic microorganisms (bacteria, fungi, yeasts, etc.) (1, 4, 19, 33) that grow on biodegradable organic matter (21, 23, 34, 42). Potentially pathogenic microorganisms (e.g., *Legionella* spp.) and microorganisms of fecal origin (e.g., *Escherichia coli*) may also find favorable conditions and proliferate in these systems (7, 10, 20, 26, 44). This bacterial biomass (generally estimated at 10^8 bacterial cells liter^{-1} in flowing water and 10^6 bacterial cells cm^{-2} in the biofilm) is the start of a complex food chain (5, 27) involving mainly protozoa and macroorganisms. There have been few studies of protozoa in water distribution networks, except in the 1960s and 1970s, when the first cases of meningeal encephalitis caused by amoebae in swimming pools occurred. More recently, Amblard et al. (1), Block et al. (4), and Servais et al. (34) have shown that relatively high densities of protozoa are present in almost all distribution system waters (from 5×10^4 to 7×10^5 liter^{-1}). These microorganisms seem to be able to multiply throughout the water treatment process, both in the granular activated carbon filters (32) and in the network itself. However, the grazing activity of these protozoa in drinking water distribution systems has not been quantified. Grazing activity is to be expected in drinking water distribution systems as in the microbial food webs in natural aquatic systems such as lakes (8), oceans (36), and rivers (14). This means that a fraction of the bacterial biomass produced is removed by protozoa. It therefore becomes important to consider protozoa not only as a reservoir of potential pathogens (2, 6, 22, 42) but also as predators that help control bacterial biomass growth and accumulation in drinking water distribution systems.

This study was carried out to obtain direct proof of protozoan activity by counting protozoa in water and biofilm samples taken from drinking water distribution systems and by estimating their grazing activity. Indirect proof was also sought by measuring the disappearance of an allochthonous microorganism, *E. coli*, experimentally introduced into two pilot networks, one having a relatively high protozoan density (network fed with O_3/GAC water [water treated with O_3 and filtered on granular activated carbon]) and the other a low density of protozoa (network fed with nanofiltered water).

MATERIALS AND METHODS

Experimental distribution systems. Each distribution system (A and B) was composed of three loops of pipes in series (Fig. 1) (39). Each loop was 31 m long and 100 mm in internal diameter (cement-lined cast iron pipes), with a water velocity of around 1 m s^{-1} and an average hydraulic residence time of 12 h loop^{-1} (i.e., 36 h for the whole distribution system). The average hydraulic residence time was set at 24 h loop^{-1} for the experimental injection of *E. coli* into the GAC water-supplied network. The linear part of each loop included 21 special devices for exposing test coupons of polyvinyl chloride (PVC; around 2-cm^2 wetted surface area) or polyurethane foam (around 1 cm^3) for bacterial and protozoa biofilm samplings. Experiments carried out in the nanofiltered

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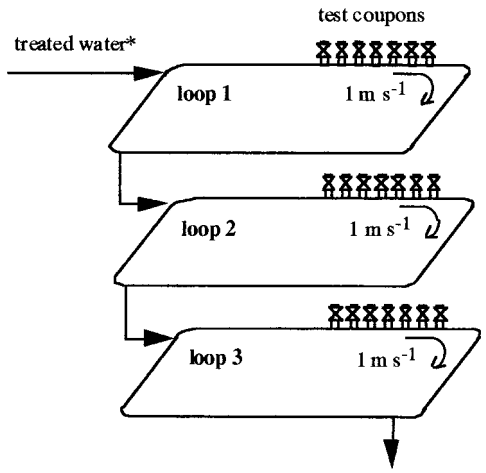

FIG. 1. Schematic representation of one of the two experimental distribution systems (*, GAC or nanofiltered water).

water- or GAC water-supplied network shown that bacterial densities of biofilm developed on PVC and cement coupons are similar (13, 29). Therefore, results obtained for PVC coupons were extrapolated for cement pipe walls.

Experimental distribution system A was continuously fed with mineralized GAC water from the drinking water treatment plant of the city of Nancy, France, and experimental distribution system B was continuously fed with mineralized nanofiltered water produced by a microfiltration followed by nanofiltration of GAC water, using two spiral-type membranes (capacity = 100 liters h^{-1}). Systems A and B were both fed continuously for at least 2 months before experimental contamination with *E. coli* and for at least 4 months more before the protozoa were counted.

Contamination of the experimental networks with *E. coli*. A suspension of an environmental strain of *E. coli* isolated from a drinking water distribution system was prepared as described by Fass et al. (10). *E. coli* was starved for 24 h at 22 ± 2°C in autoclave-sterilized distribution water taken from experimental network A or B and concentrated by centrifugation at 15,000 × *g* for 20 min at 20°C. The resulting bacterial suspension was injected into the experimental distribution systems. Single massive injections of around 10^{11} to 10^{12} *E. coli* cells (of which 20% were culturable) were introduced in less than 3 min into the first loop of each distribution system, using a peristaltic pump.

DOC and BDOC. Dissolved organic carbon (DOC) was measured with a carbon analyzer (O.I. Corporation model 700 TOC analyzer) after filtration through washed 0.45-μm-pore-size filters (Millipore no. 84908). The biodegradable fraction of organic matter (BDOC) was measured by a method adapted from Joret et al. (18).

Bacterial cell counting techniques. Bacteria in the biofilm and in the water samples were counted by three techniques. The bacteria on the PVC coupons were first removed by sonication for 2 min (cone probe, Vibra Cell 72401; power, 2 W) in 25 ml of bacterium-free distilled water.

The total cell count was made by staining the bacterial cells with a DNA fluorochrome (4′,6 diamidino-2-phenylindole [DAPI]), and the stained cells were counted by epifluorescence microscopy (30). The number of culturable bacteria was determined by the pour plate technique using standard nutrient agar without glucose (3 and 15 days at 22 ± 2°C) (38).

Coliforms were counted by a membrane filtration technique (Afnor NF T 90-414) with 2,3,5-triphenyl-tetrazolium chloride [TTC]-Tergitol 7 agar medium.

Protozoan cell counting techniques. The experimental networks contaminated with *E. coli* were cleaned (high chlorination during 7 days) and fed for 4 months with GAC water (network A) or nanofiltered water (network B). At the end of this time, the protozoa in the water were counted. Water samples were collected in 1,000-ml sterile bottles containing fixative (ice-cold glutaraldehyde; Sigma no. G7776; 2%, final concentration).

(i) Free ciliates, naked amoebae, or thecamoebae. A 500-ml sample of fixed water was concentrated by slow filtration through a polycarbonate filter (DMF no. 111 159; pore size, 0.8 μm). The filter was then placed in a round-bottom flask containing 5 ml of protozoon-free distributed water and mixed for 10 s in order to detach the protozoa from the filter. Two aliquots (50 μl) of subsamples were then examined in Nageotte cells (Prolabo no. 05 711 124) with a microscope under transmitted light (magnification, ×200). For each group of protozoa, a mean of the two enumerations was calculated.

(ii) Free flagellates. A 500-ml sample of fixed water was filtered through a black polycarbonate filter (DMF no. 111 159; pore size, 0.8 μm), which was then placed in 5 ml of dye (primulin; Sigma no. P7522; 250 μg $liter^{-1}$) in 0.1 M Trizma buffer (Sigma no. T3253) for 45 min. Excess dye was removed by filtration, and the filter was rinsed with 25 ml of 0.1 M Trizma buffer and mounted between a slide and glass coverslip with a drop of buffered glycerine (Diagnostics Pasteur no. 74921). The filters were examined under UV light with an epifluorescence microscope (Olympus; magnification, ×400). Thirty fields were counted, and the results were expressed as flagellates per liter.

(iii) Protozoa on the PVC coupons. Protozoa on the PVC coupons were removed by scraping the surface with a scalpel and rinsing the coupons with 3 ml of ice-cold glutaraldehyde (2%, final concentration). Two 50-μl aliquots were observed in Nageotte cells under transmitted light (magnification, ×200). Protozoan cells (see above) were counted and expressed as cells per square centimeter.

Protozoan grazing rate estimates in water. Grazing activity was based on the contact between protozoa and fluorescently labeled beads. The technique was adapted from Pace and Bailiff (28), Sherr et al. (35), and Sherr and Sherr (37). A stock suspension of tracer beads was prepared from a concentrated suspension of 0.5-μm-diameter fluorescent microspheres (Osi no. 15700). The bead stock suspension was treated with bovine serum albumin (Sigma no. A4378; 0.5 mg ml^{-1}) to avoid clumping (28); 50 μl of this suspension was passed through a 0.2-μm-pore-size filter (DMF no. 130210), and the beads were counted under a microscope. The microsphere suspension was then added to 3,000 ml of the water sample in an acid-washed glass round-bottom flask to give a final bead concentration of 25% of the total bacterial cell density. This solution was incubated for 30 h at 22 ± 2°C without mixing. Preliminary studies showed that no beads were ingested by the protozoa during the first 60 min. Samples (100 ml) were therefore taken every 2 h for 30 h, and ingestion was stopped by adding ice-cold glutaraldehyde (2%, final concentration). The uptake of beads by the protozoa was determined by filtering 100-ml samples of water through a black polycarbonate filter (pore size, 0.8 μm). Protozoa retained by the filter were stained with 5 ml of DAPI (Sigma no. D9542; 0.5 μg ml^{-1}) for 10 min, and the DAPI was removed by filtration. The filter was rinsed with 50 ml of bacteria-free distilled water and mounted between a slide and glass coverslip with a drop of buffered glycerine. For each filter, 35 to 40 individual protists (ciliates, flagellates, naked amoebae, or thecamoebae) were observed under UV light, and ingested beads were counted under blue light at a magnification of ×1,000.

The number of beads per protozoan initially increased linearly and then leveled off due to egestion. The slope of the linear portion of the uptake curve was taken as the rate of bead uptake by the protists of interest (35, 37). The results were expressed as beads per protozoan per hour.

Protozoan grazing rate estimates in biofilm. Seven coupons of polyurethane foam that had been in loop 1 of distribution system A for 5 months were removed. Each was placed in a 20-ml suspension of microspheres (10^7 beads ml^{-1}, about 97% of the total abundance of bacterial cells) and incubated at 22 ± 2°C for 17 h. Preliminary studies showed that bead uptake by attached protozoa began after 5 h of incubation and that beads were egested after 23 h. One coupon of polyurethane foam was squeezed after 5, 6.5, 8, 9.5, 11, 14, and 17 h of incubation to extract protozoa, and the coupon was rinsed five times with ice-cold glutaraldehyde (2%) to prevent egestion of the beads. The volume of extract was brought to 3 ml and added to 20 ml of the microsphere incubation suspension. Duplicate 50-μl subsamples were placed in Nageotte cells, which were scanned with transmitted light at a magnification of ×200. Each ciliate observed (on average, 30 to 35 individuals per 50 μl) was inspected under blue light, and the

TABLE 1. Water characteristics at the inlet of the distribution systems fed with GAC water (network A) or nanofiltered water (network B)

Network	Avg (SD)							
	No. $liter^{-1}$							Temp (°C)
	Total bacterial cells	Culturable bacteria (CFU)		Total protist cells	C (mg)		Total chlorine (mg of Cl_2)	
		3 days	15 days		DOC	BDOC		
A (n = 3)	1.4×10^8 (6.3×10^7)	7.8×10^4 (7.6×10^3)	6.0×10^6 (1.5×10^6)	3.2×10^4 (1.5×10^3)	2.3 (0.6)	0.7 (0.7)	0	21.8 (1.3)
B (n = 13)	5.0×10^4 (1.5×10^5)	3.0×10^3 (3.0×10^3)	4.9×10^4 (1.4×10^5)	0[a]	0.21 (0.05)	<0.1	0	22.3 (1.8)

[a] 0, not detectable.

TABLE 2. Water and biofilm characteristics in the first loop of the distribution system fed for 2 months with GAC water (network A) or nanofiltered water (network B)

First loop of network:	Avg (SD [$n = 3$])[a]								
	No. liter^{-1} or cm^{-2}					C (mg liter^{-1})		Total chlorine (mg of Cl_2 liter^{-1})	Temp (°C)
	Total bacterial cells	Culturable bacteria (CFU)		Coliforms (CFU)	Total protist cells	DOC	BDOC		
		3 days	15 days						
A									
Water	3.6×10^8 (4.7×10^7)	3.2×10^4 (2.3×10^4)	2.2×10^6 (1.3×10^6)	0	1.8×10^5 (8.2×10^4)	1.7 (0.6)	0.55 (0.6)	0	24.9 (1.8)
Biofilm	4.3×10^7 (3.5×10^5)	5.9×10^3 (6.4×10^3)	3.3×10^5 (3.0×10^4)	0	1.4×10^3 (86)	—	—	—	—
B									
Water	5.3×10^7 (4.8×10^6)	1.3×10^4 (9.1×10^3)	1.7×10^5 (5.9×10^4)	0	0	0.3 (0)	0	0	24.5 (1.6)
Biofilm	7.1×10^6 (3.6×10^5)	2.4×10^4 (3.5×10^2)	1.6×10^5 (1.3×10^4)	0	0	—	—	—	—

[a] 0, not detectable; —, not measured.

ingested beads were counted (magnification, ×400). The bead uptake rate was determined as described above.

RESULTS

Microbial characteristics of the drinking water distribution systems. Two parallel experimental distribution systems were continuously fed with GAC water (network A) or with nanofiltered water (network B) (Table 1). Their nutritive and microbiological characteristics were very different because nanofiltration removed most of the protistan and bacterial cells (the low density of bacteria counted may be attributed to a biofilm developed in pipes between nanofiltration unit and the network) as well as much of the dissolved organic matter (DOC and BDOC).

The two experimental systems were well colonized by bacteria after 2 months (Table 2) (up to 3×10^8 bacterial cells liter^{-1} and up to 4×10^7 bacterial cells cm^{-2} in biofilm). Bacteria multiplied significantly in the nanofiltered water network in spite of the lower concentration of biodegradable organic matter (below the sensitivity limit of the BDOC method, i.e., 0.1 mg of C liter^{-1}) compared to the GAC water network (0.55 mg of C liter^{-1}). However, the decrease of 30% in the size of cells and 1 log in the number of bacterial cells suggest that the total biomass was slightly lower in the nanofiltered water network than in the GAC water system. By unit of pipe length, the bacteria in the biofilm were on average three times more abundant than in the water in the two networks. Protists were easily counted in the GAC water-supplied network (up to 10^5 liter^{-1} in the water and 10^3 cm^{-2} in the biofilm), but no protistan cells were detected in the nanofiltered water network.

Diversity of protozoa in the network. Densities of protozoa were determined in the two networks after 4 months of feeding. No protozoa were detected in the three loops of the nanofiltered water network, but four groups of protists were identified in the water of the GAC water network: ciliates (e.g., *Colpidium campilum* and scuticociliates), thecamoebae (e.g., *Trinema lineare* and *Euglipha* sp.), and naked amoebae and flagellates (e.g., *Entosiphon sulcatum* and dinoflagellates) (Table 3). The water in loop 1 (network A) contained mainly flagellates (93% of the total protozoan abundance), followed by ciliates (1.8%), thecamoebae (1.6%), and naked amoebae (1.1%). Naked amoebae and flagellates were not detected in the biofilm, where ciliates were present in a greater numbers than thecamoebae. As the water flowed from loop 1 to loop 3, with a mean age in the system increasing from 12 h to 36 h, the total numbers of protozoa in the water and in the biofilm generally decreased. This decrease was not significantly correlated with the total number of bacterial cells in the water or the biofilm.

TABLE 3. Average density of protozoa and bacteria in the water and biofilm of the distribution system fed for 4 months with GAC water (network A)[a]

Sampling point	Avg density (SD [$n = 3$]), liter^{-1} or cm^{-2}				
	Ciliate	Thecamoebae	Amoebae	Flagellate	Bacterial cells
GAC water entering the network	695 (61)	1,170 (34)	548 (37)	3×10^4 (1.5×10^8)	2.6×10^8 (1.9×10^7)
Loop 1					
Water	3,300 (360)	2,870 (38)	2,100 (72)	1.67×10^5 (8×10^4)	3.6×10^8 (4.7×10^7)
Biofilm	765 (10)	673 (73)	0[a]	0	4.3×10^7 (3.5×10^5)
Loop 2					
Water	936 (180)	1,380 (180)	110 (6)	1×10^5 (6×10^4)	3.9×10^8 (5.2×10^7)
Biofilm	817 (53)	584 (22)	0	0	1.4×10^7 (7.3×10^5)
Loop 3					
Water	727 (180)	1,090 (290)	0	2.5×10^4 (4×10^3)	2.9×10^8 (1.8×10^7)
Biofilm	322 (52)	568 (30)	0	0	1.1×10^7 (0)

[a] Hydraulic residence time, 12 h loop^{-1}; temperature, 25 ± 1.5°C. Detection limits: flagellate, 1,480 liter^{-1} or 15 cm^{-2}; ciliate, thecamoebae, and naked amoebae, 100 liter^{-1} or 15 cm^{-2}. 0, not detectable.

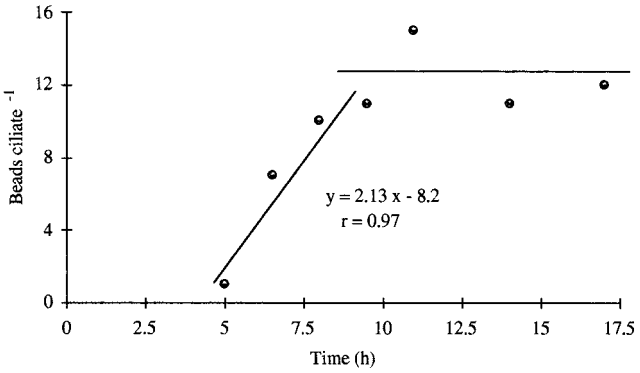

FIG. 2. Estimation of the grazing activity of biofilm ciliates with tracer beads (GAC water-supplied network, loop 1).

Microscopic examination of the protozoa in the water of network A (loops 1 to 3) showed many bacteria in the food vacuoles as a result of the grazing activity of the protozoa, but this activity was not confirmed by laboratory measurements of the ingestion rate of fluorescently labeled beads (0.5 μm in diameter). Protozoa taken from the water did not ingest free labeled beads even after 30 h of incubation. This lack of ingestion of tracer beads may be because the protozoa did not recognize beads as being potential prey or because the protozoan digestive vacuoles were full of bacteria and so did not ingest beads.

In contrast, the ciliates sampled from biofilm (polyurethane coupons) in the three loops of network A showed significant grazing activity. They began ingesting beads after 5 h, and the number increased linearly from 5 h to 10 h ($r = 0.97$) (Fig. 2); the specific ingestion rate was 2 beads per ciliate per h. Assuming that ciliates (i) removed beads and bacteria at the same, constant rate throughout the study and (ii) had the same activity in the polyurethane coupons as in the biofilm on the pipe walls, we may calculate the grazing activity of protozoa in each loop of network A. The ciliates in the biofilm could remove around 1,630 bacteria h^{-1} cm^{-2} (loop 1) to 686 bacteria h^{-1} cm^{-2} (loop 3). In the GAC water system, the contribution of protozoa to the biofilm removal was low: only 0.003% of the fixed bacterial abundance could be ingested per h by ciliates present in 1 cm^2 of biofilm.

Behavior of *E. coli* added to the distribution system. The behaviors of the *E. coli* cells experimentally added to networks A and B were compared, taking into account that a key difference between the two systems was the protozoan density (up to 10^5 $liter^{-1}$ in network A and not measurable in network B). Figure 3 shows the total number of *E. coli* (CFU in water plus biofilm) as a function of the number of hydraulic residence times, allowing a direct comparison of the two systems working at different hydraulic residence times (network A at 24 h and network B at 12 h). The first loop of each network was contaminated by single injections of 10^{11} CFU (network A) and 4×10^{10} CFU (network B).

The *E. coli* cells in the GAC water (network A, containing protozoa) disappeared much faster than those in nanofiltered water (network B, protists undetectable). The ability of *E. coli* to colonize the network B was unexpected, because the concentration of bioavailable organic matter was very low and there were viable autochthonous bacteria. Indeed, the slope of the *E. coli* decay curve in network B (0.001 h^{-1}) was lower than the theoretical dilution rate ($D = 0.083$ h^{-1}), suggesting that *E. coli* adapted and grew in the distribution system. This major difference in the behavior of *E. coli* in the two networks A and B may be indirect proof of the high and low (not detectable) protozoan grazing, respectively.

DISCUSSION

Drinking water distribution systems are constantly exposed to an inflow of microorganisms (bacteria, fungi, protozoa, algae, nematodes, etc.) (1, 4, 11, 33). Some of the viable organisms can adapt to this oligotrophic environment and form a stable ecosystem. A nanofiltered water-supplied network had a microbial ecosystem in the water phase (5×10^7 bacterial cells $liter^{-1}$), and in the biofilm (7×10^6 bacterial cells cm^{-2}), but this ecosystem seemed to include only bacteria because no protozoa were detected in the nanofiltered water network (water plus biofilm). The apparent absence of predatory organisms could also be due to (i) the filtration (pore size, 10 nm) of the water entering the system removing protists and (ii) a lack of protozoan growth because of the very low bacterial biomass (prey), which could have been under the threshold level that would permit protozoan maintenance and/or growth. The low bacterial growth resulted from the removal of more than 90% of the organic carbon by nanofiltration.

In contrast, the conventionally treated (GAC) water network allowed the establishment of a much greater trophic food web. The ecosystem in the distribution system had at least two fractions: bacteria (3×10^8 cells $liter^{-1}$ in water and 4×10^7 cells cm^{-2} in biofilm) and protozoa (10^5 cells $liter^{-1}$ in water and 10^3 cells cm^{-2} in biofilm) as previously shown by Amblard et al. (1) and Servais et al. (34). The GAC water-supplied system contained flagellates, ciliates, naked amoebae, and thecamoebae, with percentages similar to those reported by Amblard et al. (1). The biofilm contained only two groups of protozoa, thecamoebae (48%) and ciliates (52%).

Only the ciliates at the solid-liquid interface of the GAC water-supplied network had measurable grazing activity via a laboratory test, estimated at 2 bacteria $ciliate^{-1}$ h^{-1}. The protozoa in the water phase of the GAC water-supplied network had no detectable grazing activity. These results could be due to the inadequacy of the laboratory test for free-living protozoa in drinking water distribution system and/or the greater ability of protozoa to ingest fixed bacteria instead of planktonic bacteria. The grazing activity of fixed protozoa in drinking water

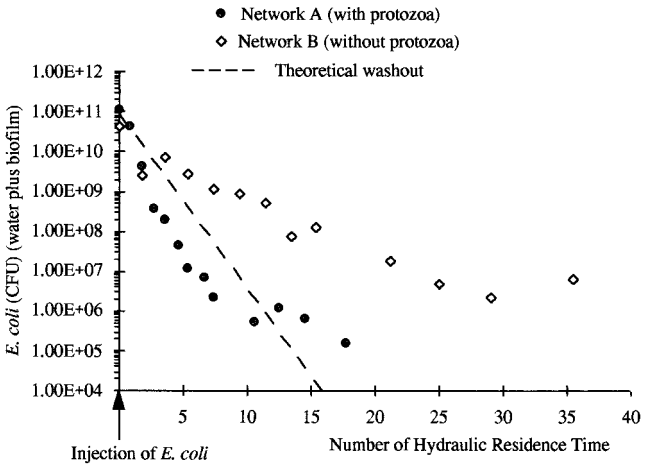

FIG. 3. Density of *E. coli* in the first loop of the GAC water-supplied network A (hydraulic residence time = 24 h $loop^{-1}$) and nanofiltered water-supplied network B (hydraulic residence time = 12 h $loop^{-1}$).

biofilms has never been published. The grazing activity of planktonic ciliates is greater in an aquatic environment and varies from 380 to 1,095 bacteria ciliate^{-1} h^{-1} in marine water (36), from 5 to 80 bacteria ciliate^{-1} h^{-1} in river water (14), and from 6 to 5,910 bacteria ciliate^{-1} h^{-1} in lake water (8).

An indirect indication of protozoan ingestion of bacteria was obtained by measuring the fate of *E. coli* experimentally introduced into two distribution systems. As previously described (10) a transient contamination of *E. coli* was introduced into the networks, but unexpectedly, the *E. coli* was removed more rapidly from the GAC water-supplied network than from the nanofiltered water-supplied network. This difference was assumed to be due to the protozoan densities in the two pipe loops.

As pointed out by Sime-Ngando et al. (41), the number of protozoa seen under the microscope, and consequently the estimation of their densities, is influenced by the fixative and concentration. The efficiency of the scraping method used to remove protozoa from the coupons is probably questionable, so that the numbers reported are underestimates of the true densities.

The factors that determine the rates at which protozoa ingest bacteria are poorly understood, and there is considerable variation in the degree to which different types of protists discriminate between surrogate food particles and natural food preys (17, 24). Protozoa may prefer motile bacteria to beads (12, 25, 35, 40), and the size of bacteria or beads is also an important factor that can affect the grazing rate of protozoa (31).

Grazing activity also depends on the physiological state of the protozoa, which can be sensitive to physical stress (9) and is linked to the digestion rate, which decreases greatly for small bacteria because a more elaborate digestion is needed to extract of all the nutritive molecules from small prey (12). Chemotaxis (capacity to detect specific chemical products and to migrate [16]), which is an essential behavioral feature of ciliates and flagellates (3, 38), is also affected by the history and the physiological state of the prey species (44). The specific ingestion rate measured in the biofilm may be overestimated because of the presence of more prey than in the usual medium. Iriberri et al. (15) have shown that high densities of prey promote greater specific clearance rates for two species of ciliate (*Uronema* sp. and *Colpoda inflata*).

Although the accuracy of the protozoan ingestion rate estimated in this study is questionable, the method is quite innovative and gives an initial assessment of the ecological significance of both protozoan densities and grazing activities in a drinking water distribution system. Unlike knowledge of the protozoa in natural ecosystems (8, 14), the protozoan populations in drinking water networks have been described only recently.

Despite the need to optimize the methods used, this study shows (i) that the nanofiltration process limits the protozoan component of the food chain by limiting the number of prey organisms and by removing protozoa in the feeding water and (ii) that in the GAC water network, which contained a varied ecosystem, the grazing activity contributed minimally to the control of the attached bacterial population.

ACKNOWLEDGMENTS

This work was carried out as part of a larger research program coordinated by the Centre International de l'Eau de Nancy (NANCIE) and funded by the Compagnie Générale des Eaux (Paris, France), the Communauté Urbaine du Grand Nancy (Nancy, France), the Syndicat des Eaux de l'Ile de France (Paris, France), the Office National de l'Eau Potable (ONEP Maroc), the Agence de l'Eau Seine-Normandie (Paris, France), Pont à Mousson S.A. (France), and NANCIE.

We thank Laurent Probst (NANCIE) for maintaining the experimental distribution network, M. Mignot (Laboratoire de Biologie Comparée des Protistes, Clermont Ferrand) for identifying some protozoa in the distribution system, and Vincent Gauthier (NANCIE-LSE) for helpful discussions.

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