Applied and Environmental Microbiology, Nov. 1996, p. 4014–4018 Vol. 62, No. 11
0099-2240/96/$04.00+0
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Effect of Growth Conditions and Substratum Composition on the Persistence of Coliforms in Mixed-Population Biofilms

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Received 18 December 1995/Accepted 27 August 1996

Laboratory reactors operated under oligotrophic conditions were used to evaluate the importance of initial growth rate and substratum composition on the long-term persistence of coliforms in mixed-population biofilms. The inoculum growth rate had a dramatic effect on the ability of coliforms to remain on surfaces. The most slowly grown coliforms ($\mu = 0.05$/h) survived at the highest cell concentration. Antibody staining revealed that *Klebsiella pneumoniae* existed primarily as discrete microcolonies on the surface. Both coliforms and heterotrophic plate count bacteria were supported in larger numbers on a reactive substratum, mild steel, than on polycarbonate.

Over the past decade, drinking water utilities have reported unexplained occurrences of coliform bacteria in finished drinking water, termed regrowth. After thorough investigation of the potential source of these bacteria, the opinion has been that these organisms must be growing on the pipe walls of the distribution system at the expense of the low concentrations of carbon present in the water, detaching from the surface, and being detected as suspended bacteria. It is likely that biofilm growth is responsible for the presence of the coliforms, because there is insufficient usable organic carbon present to support the proliferation of suspended cells (8, 13, 35). Although coliforms are generally perceived to be copiotrophic and mesophilic, isolates from drinking water are capable of growth in batch cultures under oligotrophic conditions and temperatures relevant to distribution systems (6). In a limited number of cases, investigators have reported coliforms in association with deposits from full-scale distribution system materials (11, 19). Locating these organisms has been difficult even in systems plagued with coliform regrowth problems, presumably because they are present nonuniformly on the surfaces.

The drinking water industry is interested in knowing what water quality parameters and environmental conditions lead to coliform growth in biofilms. Investigators have had limited success in establishing these organisms in mixed-population biofilms for extended periods (1). Therefore, the systematic study of conditions in laboratory and pilot systems that result in the presence of these bacteria in the field has been impossible.

The research described here was carried out to determine conditions that allowed indicator bacteria to successfully colonize and persist in laboratory reactors operated under oligotrophic conditions similar to those in municipal distribution systems. These conditions were selected on the basis of an extensive literature review (3) and discussions with drinking water utilities. The initial growth rate was investigated, since oligotrophic environments favor slowly growing organisms (2). The type of material for colonization was also deemed important, because past research has shown that coliforms were found in association with iron tubercles in pipes (11, 19).

Because conditions in the laboratory reactors could be easily controlled, the distribution of coliforms in biofilms established under low-nutrient conditions was also investigated. Although the coliform distribution in dual-species biofilms (31) or systems fed sterile water over a short period (33) has been investigated, no information is available on their distribution in biofilms under long-term competition from autochthonous heterotrophs. These observations would be helpful in explaining the inability of utilities to locate uniformly distributed coliforms on pipe surfaces (8, 19).

MATERIALS AND METHODS

Reactor design and operation. Parallel polycarbonate annular reactors were used to simulate the shear stresses, temperature, and substrate concentrations found in a drinking water distribution system. The reactors consist of a stationary outer cylinder with 12 removable flush-mounted slides for biofilm sampling (Fig. 1). The slides used in these experiments were either an inert plastic (polycarbonate) or a material subject to corrosion (mild steel, also known as carbon steel). The rotation of the inner cylinder was set to simulate the shear stress found in a 4-in. (ca. 10-cm) pipe with a fluid velocity of 0.3 m/s (1 ft/s). Draft tubes in the inner cylinder facilitate mixing so that the reactors can be modeled as continuously stirred tank reactors. The residence time was set at 2 h (dilution rate of 0.5/h). This dilution rate was most favorable in allowing coliforms to persist in mixed-population biofilms (4) and is also shorter than the growth rate of the organisms (6). Because planktonic growth was minimal, any increased number of bacteria in the effluent of the reactor compared with the influent is expected to be the result of detached biofilm bacteria. The influent to the reactor consisted of separate feeds of nitrate and phosphate (100 μg/liter each), a carbon source (250 μg of C per liter), and dilution water. The carbon source consisted of equimolar concentrations on the basis of carbon of acetate, sodium benzoate, propionaldehyde, parahydroxybenzoic acid, and ethanol. These compounds were chosen because they represent major classes of compounds found in drinking water (33) that are relatively nonreactive with disinfectants (16). All chemicals were supplied by Sigma Chemical Co. Dilution water was Bozeman tap water that was passed in an upflow mode through two columns. The first column contained granular activated carbon (12- by 40-mesh Nuchar; Westvaco) to remove chlorine, and the second was filled with biologically activated carbon from a full-scale water treatment plant (City of Laval, Quebec, Canada) to decrease the concentrations of naturally occurring organic compounds that may support microbial growth. The empty-bed contact time for each filter was 15 min. Effluent from these columns consistently contained <25 μg of assimilable organic carbon per liter as determined by the method of van der Kooij et al. (34) and modified by LeChevallier et al. (21). The dilution water also contained a substantial number of heterotrophic bacteria (ca. 10^4 CFU/ml) that could colonize the annular reactor surfaces and essentially no coliforms (<1 CFU/ml). The nitrogen-phosphorous and substrate feed supplies were made with the carbon column effluent in large glass carboys and autoclaved before use. All influent materials were supplied to the reactors via peristaltic pumps (Cole-Parmer).

The temperature was maintained at 20°C by immersing the reactors in a water bath attached to a Fisher Isotemp recirculating cooling device.

Before each experiment, the reactors were disassembled, completely cleaned, reassembled, and autoclaved, with the exception of the mild steel slides. Because autoclaving resulted in excessive corrosion, the mild steel slides were sterilized

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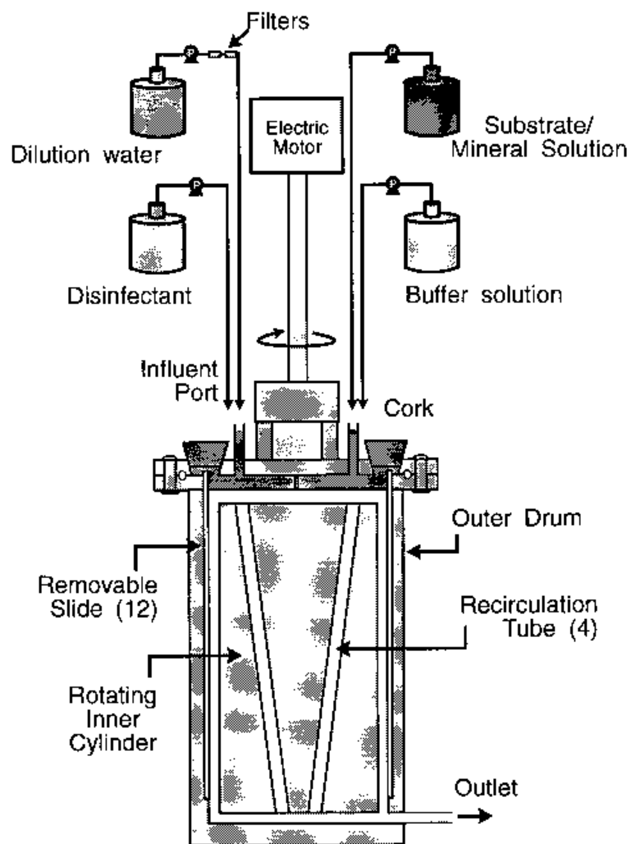

FIG. 1. Schematic of the laboratory annular reactor.

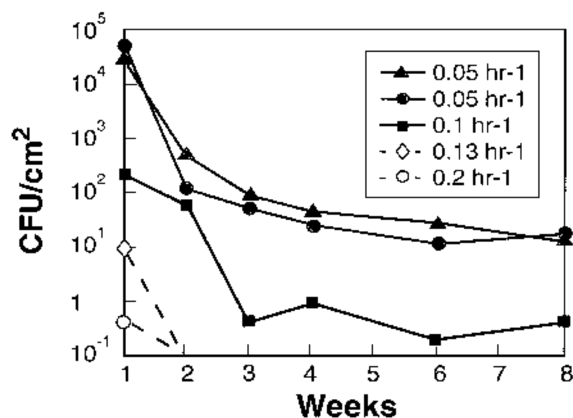

FIG. 2. Impact of initial growth rate on the long-term persistence of coliforms in mixed-population biofilms on a polycarbonate substratum. Coliforms grown in chemostats with a residence time of 20, 10, 7.5, or 5 h were used to inoculate the reactors.

with UV light for 45 min before being installed in the reactor. All tubing was replaced, and the pumping rates were recalibrated.

Coliforms. The coliforms were originally isolated from drinking water distribution systems and were identified with API 20 E strips (Analytab Corp.) to be *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes*, *E. cloacae*, and *Escherichia coli*. To minimize the chance of genetic or phenotypic drift, the organisms were streaked for isolation and then frozen in 20% glycerol–2% peptone at −70°C. The frozen stocks were used to start each experiment.

The organisms were grown in batch cultures before each experimental run in 100 ml of sterile column effluent containing 5,000 μg of C per liter of substrate and 1,000 μg each of nitrate and phosphate per liter. Incubation was carried out at 20°C in a shaking incubator for 48 h.

Chemostats. A total of four chemostat residence times (5, 7.5, 10, and 20 h) were selected to determine the impact of initial coliform growth rate on the ability of coliforms to colonize and persist long term in mixed population biofilms. For the substratum experiments, a residence time of 20 h was used. The chemostats were aerated with filtered air supplied by an aquarium pump. A 1-ml sample of each coliform culture was added to duplicate sterilized 500-ml chemostats fed the same concentrations of substrate and nutrients as the starter cultures; the total flow was split between the substrate and nitrate-phosphate solutions. A steady-state population was reached within three residence times and assessed by measuring organism numbers on mT7 and/or R2A agar (Difco). Individual colonies were identified. All five organisms were routinely found in the chemostat effluent. Typical cell numbers were 10^5 CFU/ml.

Reactor inoculation. The entire contents of the chemostats were rapidly pumped to the parallel empty reactors while the inner drums were rotating. The chemostat feed supply was transferred to annular reactors run at the same dilution rate as the chemostat for one residence time. The chemostat feed was then terminated, and the reactors were operated as previously described. The dilution water acted as the source of heterotrophic organisms.

Fluid sampling. Influent and effluent samples were collected in sterile tubes from both reactors at each sampling time (weekly for the first 4 weeks and then every other week for 1 month). These were analyzed for coliform numbers and heterotrophic plate counts.

Culturable bacterial numbers were obtained by the spread plate technique. Samples were homogenized by the method of Camper et al. (5), diluted appropriately, and placed on triplicate mT7 (for coliforms) or R2A plates (for heterotrophs). Incubation was carried out at 35°C for 24 h (for coliforms on mT7) or at room temperature for 7 days (for heterotrophs on R2A).

Biofilm sampling. At the sampling times described above, slides were removed from the reactors for biofilm analyses and replaced with a sterile slide. Two slides per sampling time were used to determine the reproducibility of the results; this was reduced to one per sampling time when it was found that there was no significant difference in bacterial numbers. Sampling continued for 8 weeks or until no coliforms were detected. Deposits on the slides were scraped with a flame-sterilized utility knife into 10 ml of sterile water in a 100-ml beaker. The contents of the beaker were homogenized, and bacterial numbers were determined as above. Results were reported as the number of CFU per square centimeter of slide surface.

In some instances, slides were removed, the biofilm was fixed in 1% paraformaldehyde, and the cells stained with 5 mg of propidium iodide per liter for 15 min. The biofilm was then exposed to skim milk as a blocking agent to reduce nonspecific staining, rinsed with phosphate-buffered saline, and incubated in the presence of a monoclonal antibody produced against *K. pneumoniae*. Following an additional rinse, a fluorescein-conjugated antibody directed against the monoclonal was applied. The slides were observed in an Olympus BH-2 microscope attached to a Bio-Rad MRC 600 confocal laser system. The images produced indicated total cells by a red fluorescence and antibody-labeled cells by a green emission.

At the end of the 8-week experiments, the biofilm on the inner drum of the annular reactor was removed with a Teflon cell scraper directly into 100 ml of sterile water. The cells were dispersed by homogenization, and the coliforms and heterotrophs were enumerated by plate counts as for the fluid samples. In some instances, replacement slides from the first few weeks were removed and the biofilms were analyzed.

Data analysis. Plate count data were reported as the mean of the three plates from the appropriate dilution on a per-milliliter (fluid) or per-square-centimeter (biofilm) basis. Duplicate slides were removed and analyzed for the number of culturable heterotrophs and coliforms. The standard error between slides in both bacterial counts was less than 10%, indicating good replication at different locations within the reactor.

RESULTS

Importance of initial growth rate. In replicate experiments with parallel reactors inoculated with coliforms from chemostats with a dilution rate of 0.20/h, the indicator bacteria were eliminated within the first week after exposure to heterotrophs in the water (Fig. 2). When the initial growth rate was decreased to 0.13/h, coliforms were not detected after the first week. If the coliforms were initially cultured at a growth rate of 0.10/h, they persisted for the entire 8-week period, albeit at very low levels. The lowest initial growth rate of 0.05/h enabled the coliforms to successfully compete and remain in the biofilms for the entire experimental run time at a level approximately 1 log unit higher than observed with the 0.10/h growth rate. In both cases, the number of indicator organisms declined to a pseudo-steady-state level between 3 and 4 weeks after

inoculation. In all experiments, the levels of heterotrophs in the biofilms were constant at ca. 10^6 CFU/cm^2 regardless of the sampling time.

The suspended coliforms followed the same trend with initial growth rate as did the biofilm bacteria. Planktonic cell counts were approximately 1/100 ml and 10/ml for the 0.10/h and 0.05/h inocula, respectively, and were not detected in the 0.20/h and 0.13/h runs. Heterotrophs in the effluent were always present at concentrations of approximately 10^5 CFU/ml.

Comparisons of coliform colonization potential in reactors with mild steel were also conducted with bacteria at an initial growth rate of 0.10 and 0.05/h. Bacterial numbers in the biofilms were at least twice as large when the reactors were inoculated with bacteria grown at the lower growth rate.

Coliform distribution in biofilms. Destructive sampling at the end of two experiments was conducted to determine if the coliform numbers on the inner drums of the annular reactors were similar to those found on the original polycarbonate slides. In one experiment, parallel reactors with coliforms grown at 0.10/h and 0.05/h were operated. Organism numbers from the inner drums and the slides taken from the last sampling time (week 8) were within 1 standard error, suggesting that there was no preferential association with the inner or outer cylinder.

Concurrently, the coliforms isolated from the inner drums were identified and their metabolic profiles were compared with those found in the chemostat effluent containing all five coliforms used to inoculate the reactors. Not all coliforms were found in the reactors at the end of the experiment. Of 18 colonies isolated from the 0.05/h-colonized reactor, 15 were *K. pneumoniae*, 2 were *K. oxytoca*, and 1 was *E. cloacae*. All coliforms from the 0.01/h-inoculated reactor were *K. pneumoniae*. A similar analysis was conducted on isolates from the inner drum at the end of a replicate run with a 0.05/h chemostat inoculum. Of the 35 isolates tested, 33 were identified as *E. cloacae* and the other 2 were *K. pneumoniae* and *K. oxytoca*. The metabolic profiles of the isolated biofilm organisms were identical to those of the organisms used to inoculate the reactors.

It was of interest to know how clean surfaces introduced into the reactors after a week or more of operation would be colonized with the coliforms. Slides that had been replaced with unused slides after the first sampling time (1 week after coliform addition) were sampled at the end of the experiment. The numbers of coliforms on these surfaces were similar to those from slides that had been undisturbed for the entire 8-week run time, suggesting that coliforms emanating from the biofilms could recolonize clean substrata.

A qualitative assessment of the distribution of *K. pneumoniae* was made by using images collected with time. In both reactors, samples from the first 3 weeks revealed a heterogeneous distribution of individual *K. pneumoniae* cells and nonspecifically stained heterotrophs on the surface. At week 4, the coliform was present primarily as discrete microcolonies with a few distributed individual cells. The majority of the generally stained population was present as single cells in direct contact with the substratum, but a few clumps up to 20 μm high and strings of cells were also seen. Bacteria were also found in association with small pieces of what was believed to be granular activated carbon from the biological filter. Approximately 60% of the total surface was covered with microorganisms. The overall appearance of the biofilm did not change substantially for the remainder of the experiment. *K. pneumoniae* was detected by antibody and plate counts for the entire 8 weeks and was the most abundant coliform identified at the end of the experiment.

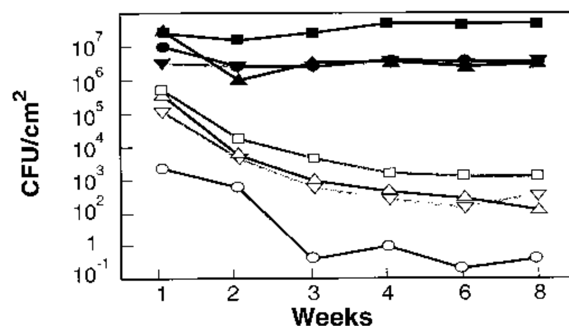

FIG. 3. Effect of substratum composition and initial coliform growth rate on heterotrophs (solid symbols) and coliforms (open symbols) in mixed-population biofilms. Symbols: ■, □, 0.05/h, mild steel; ▲, △, 0.05/h, polycarbonate; ▼, ▽, 0.1/h, mild steel; ●, ○, 0.1/h, polycarbonate.

Substratum effects. The relative importance of a reactive (mild-steel) or inert (polycarbonate) substratum for biofilm accumulation and coliform persistence was determined by using inocula from 0.10/h or 0.05/h chemostats. Substantial amounts of corrosion products accumulated on the steel slides with time. Regardless of the initial growth rate, the mild-steel reactors supported approximately 10-fold more heterotrophs and coliforms per square centimeters than did similar reactors containing only polycarbonate (Fig. 3). This increase was also seen in the reactor effluents, indicating that the increased numbers were due to an enhancement of growth rather than merely to greater accumulation on the mild steel. The increase cannot be attributed to a deficiency in iron, because no iron limitation was detected in similar experiments using the same medium with and without iron (data not shown).

Mild-steel slides taken from the reactor for sampling were replaced with polycarbonate. When the experiments were terminated, the polycarbonate slides that had been in place for 7 weeks were also analyzed for heterotrophic and coliform cell numbers. When coliform numbers on the replacement polycarbonate slides were evaluated, they were similar to those from the final mild-steel slides (98 and 110/cm^2, respectively). Heterotroph densities on the replacement slides were approximately 0.5 log unit lower than on the mild steel but were still greater than the densities on polycarbonate slides from reactors that had never been operated with mild steel. The same effect was seen on the inner drums of the reactors. The biofilms scraped from the polycarbonate replacement slides and inner cylinder contained large accumulations of corrosion products.

DISCUSSION

The initial growth rate of coliforms appears to have a long-term impact on their ability to effectively compete in a mixed-population biofilm. Regardless of the initial growth rate, the coliforms attached to the surfaces and were detected for the first week; only with the lower growth rates were the bacteria present for the entire run time. Furthermore, the organisms grown the most slowly maintained the highest population on both an inert and a reactive substratum for the entire 8-week run time. This effect was also apparent on surfaces introduced to the reactors a week or more after the initial colonization period, representing coliforms that had detached from the biofilms elsewhere in the reactor reattached to the clean surfaces. Thus, coliforms that successfully colonized the reactor surfaces were acclimated to low-nutrient conditions, either from the low-dilution-rate chemostat or from the reactor biofilm. Bacteria can change their uptake mechanisms depending

on the available substrate concentrations as a survival mechanism under low-nutrient conditions (10, 12, 17, 27). Other possibilities, as described by Roszak and Colwell (30), are the rerouting of metabolic pathways to avoid blocks from nutrient limitation, better coordination of biosynthesis to stabilize balanced growth, and the adjustment of uptake of nutrients in excess. Regardless of the mechanism, the initial growth rate has a profound influence on the long-term behavior of these bacteria. Our results may explain why it has been difficult to establish certain bacteria in biofilms when they were grown under rich nutrient conditions in batch culture. In the one reported instance in which coliform maintenance in a mixed-population biofilm has been reported (9), a drinking water isolate of *E. coli* was introduced to a continuous-flow reactor containing tiles of distribution system materials for colonization and operated at a dilution rate of 0.05/h. The practical implication of these results is that slowly growing organisms from environmental sources may be more likely to colonize surfaces under low-nutrient conditions, and that organisms shed from a biofilm may act as the inoculum for surfaces further downstream.

Even though coliform numbers were similar in replicate experiments, the predominant coliform changed from *K. pneumoniae* to *E. cloacae*. These are the two organisms most frequently communicated by utilities to be present in regrowth events. It is unclear what environmental pressures predisposed the prevalence of one organism over the other under identical conditions.

There has been an interest in describing the distribution of organisms in biofilms. In one case, the spatial distribution of classes and subclasses of organisms in drinking water biofilms, investigated with rRNA probes, revealed an intermingling of various organisms on the surface and within microcolonies (23). More specifically, two reports exist documenting the location of the indicator organism *E. coli* in biofilms; one method involved rRNA probes in a dual-population biofilm fed with benzoate (33), and the second involved fluorescent and reporter gene technology in a mixed population fed sterilized tap water (29). In our investigations, *K. pneumoniae* was most frequently present in microcolonies, which is in agreement with information obtained in other laboratory experiments with defined mixed populations (25, 31). These findings provide an explanation for the inability of chlorine to control regrowth events (7, 15, 24, 28), because microcolonies of bacteria tend to be less susceptible to disinfection (14). The general microbial population was dispersed but not uniformly distributed on the surface. The low colonization density reflects the oligotrophic conditions present in the reactor.

There are prior indications that ferrous metal surfaces support more organisms than do other materials. In pilot distribution systems with various materials, organisms growing on ferrous metal surfaces were less susceptible to free chlorine than when present on other materials (19, 20), presumably because the metal exerts a chlorine demand. Neden et al. (26) found that bacterial populations on unlined cast iron were the highest, whereas polyvinyl chloride was colonized with the smallest number. Block (1) determined that under conditions when chlorine concentrations were low or not detected, there was a progressive decrease in bacterial densities on surfaces from cast iron, tinned iron, cement lined cast, to stainless steel. Indirect evidence for the involvement of ferrous metals in coliform regrowth events was provided when corrosion control measures reduced or eliminated the regrowth problem in a distribution system (15, 22, 24).

The importance of surface material on organism numbers, including coliforms, was substantiated in our research. In the absence of a disinfectant, mild-steel surfaces were consistently colonized by nearly 10-fold more heterotrophs and 2- to 10-fold more coliforms than were polycarbonate surfaces when the reactors were operated under the same conditions. The impact extended to the effluent bacterial concentrations as well; elevated counts were found in reactors with mild steel even though only 10% of the reactor surface area is encompassed by the slides. Furthermore, the presence of mild steel affected population densities on polycarbonate surfaces in the same reactor. These surfaces supported the same numbers of bacteria as seen on the steel itself. It therefore appears that the mild-steel surface is capable of enhancing biofilm regrowth, rather than only protecting it from the action of a disinfectant.

ACKNOWLEDGMENTS

Funding was provided by the American Water Works Association Research Foundation under contract 704-91, the National Science Foundation Engineering Research Centers program under agreement EEC-8907039, and the Center for Biofilm Engineering Industrial Associates.

The assistance of Lu Goodrum with reactor operation and maintenance is greatly appreciated. We thank Shawn Handran for his help in developing the monoclonal antibody and the immunostaining. We also thank Paul Stoodley for his assistance with the confocal microscope.

REFERENCES

1. **Block, J. C.** 1992. Biofilms in drinking water distribution systems, p. 469–485. *In* T. R. Bott, L. Melo, M. Fletcher, and B. Capedeville (ed.), Biofilms—science and technology. Kluwer Publishers, Dordrecht, The Netherlands.
2. **Bouwer, E. J., and P. B. Crowe.** 1988. Biological processes in drinking water treatment. J. Am. Water Works Assoc. **80:**82–93.
3. **Camper, A. K.** 1994. Coliform regrowth and biofilm accumulation in drinking water systems: a review, p. 91–106. *In* G. G. Geesey, Z. Lewandowski, and H. C. Flemming (ed.), Biofouling and biocorrosion in industrial water systems. CRC-Lewis Publishers, Boca Raton, Fla.
4. **Camper, A. K., W. L. Jones, L. Goodrum, and J. Borkowski.** Influence of water quality parameters on biofilm growth in model drinking water distribution systems. Submitted for publication.
5. **Camper, A. K., M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters.** 1985. Evaluation of procedures to desorb bacteria from granular activated carbon. J. Microbiol. Methods **3:**187–198.
6. **Camper, A. K., G. A. McFeters, W. G. Characklis, and W. L. Jones.** 1991. Growth kinetics of coliform bacteria under conditions relevant to drinking water distribution systems. Appl. Environ. Microbiol. **57:**2233–2239.
7. **Centers for Disease Control.** 1985. Detection of elevated levels of coliform bacteria in a public water supply. Morbid. Mortal. Weekly Rep. **34:**142.
8. **Characklis, W. G.** 1988. Bacterial regrowth in distribution systems. American Water Works Association Research Foundation, Denver, Colo.
9. **Colbourne, J. S., P. J. Dennis, W. Keevil, and C. Nackerness.** 1991. The operational impact of growth of coliforms in London's distribution system, p. 799–810. *In* Proceedings of the Water Quality Technology Conference. American Water Works Association Research Foundation, Denver, Colo.
10. **Dawes, E. A.** 1976. Endogenous metabolism and the survival of starved prokaryotes. Symp. Soc. Gen. Microbiol. **26:**19–53.
11. **Emde, K. M., D. W. Smith, and R. Facey.** 1992. Initial investigation of microbially influenced corrosion (MIC) in a low-temperature water distribution system. Water Res. **26:**169–175.
12. **Geesey, G. G., and R. Y. Morita.** 1979. Capture and uptake of arginine at low concentrations by a marine psychrophilic bacterium. Appl. Environ. Microbiol. **38:**1092–1097.
13. **Haudidier, K., J. L. Paquin, T. Francais, P. Hartemann, G. Grapin, F. Colin, M. J. Jourdain, J. C. Block, J. Cheron, O. Pascal, Y. Levi, and J. Miazga.** 1988. Biofilm growth in drinking water network: a preliminary industrial pilot plant experiment. Water Sci. Technol. **20:**109–115.
14. **Huang, C. T., F. P. Yu, G. A. McFeters, and P. S. Stewart.** 1995. Nonuniform spatial patterns of respiratory activity within biofilms during disinfection. Appl. Environ. Microbiol. **61:**2252–2256.
15. **Hudson, L. D., J. W. Hankins, and M. Battaglia.** 1983. Coliforms in a water distribution system: a remedial approach. Am. Water Works Assoc. J. **75:**564–568.
16. **Jadas-Hecart, A.** 1989. Contribution à l'étude de la demande en chlore à long terme d'une eau potable: modélisation et identification de précurseurs organiques. Ph.D. thesis. Université de Poitiers, Poitiers, France.
17. **Kurath, G., and R. Y. Morita.** 1983. Starvation-survival physiological studies of a marine *Pseudomonas* sp. Appl. Environ. Microbiol. **45:**1206–1211.
18. **LeChevallier, M. W., T. M. Babcock, and R. G. Lee.** 1987. Examination and

characterization of distribution system biofilms. Appl. Environ. Microbiol. **53:**2714–2724.
19. **LeChevallier, M. W., C. D. Lowry, and R. G. Lee.** 1990. Disinfecting biofilms in a model distribution system biofilm. Am. Water Works Assoc. J. **82:**87–99.
20. **LeChevallier, M. W., C. D. Lowry, R. G. Lee, and D. L. Gibbon.** 1993. Examining the relationship between iron corrosion and the disinfection of biofilm bacteria. Am. Water Works Assoc. J. **85:**111–123.
21. **LeChevallier, M. W., N. Shaw, L. Kaplan, and T. L. Bott.** 1993. Development of a rapid assimilable organic carbon method for water. Appl. Environ. Microbiol. **59:**1526–1531.
22. **Lowther, E. D., and R. H. Moser.** 1984. Detecting and eliminating coliform regrowth, p. 323–329. *In* Proceedings of the Water Quality Technology Conference. American Water Works Association Research Foundation, Denver, Colo.
23. **Manz, W., U. Szewzyk, P. Ericsson, R. Amann, K. H. Schleifer, and T. A. Stenstrom.** 1993. In situ identification of bacteria in drinking water and adjoining biofilms by hybridization with 16S and 23S rRNA-directed fluorescent oligonucleotide probes. Appl. Environ. Microbiol. **59:**2293–2298.
24. **Martin, R. S., W. H. Gates, R. S. Tobin, R. Sumarah, P. Wolfe, and P. Forestall.** 1982. Factors affecting coliform bacterial growth in distribution systems. Am. Water Works Assoc. J. **74:**34–37.
25. **Murga, R., P. Stewart, and D. Daly.** 1995. Quantitative analysis of biofilm thickness variability. Biotechnol. Bioeng. **45:**503–510.
26. **Neden, D. G., R. J. Jones, J. R. Smith, G. J. Kirmeyer, and G. W. Foust.** 1992. Comparing chlorination and chloramination for controlling bacterial regrowth. Am. Water Works Assoc. J. **84:**80–88.
27. **Novitsy, J. A., and R. Y. Morita.** 1978. Possible strategy for the survival of marine bacteria under starvation conditions. Mar. Biol. **36:**289–295.
28. **Reilly, K. J., and J. S. Kippen.** 1983. Relationship of bacterial counts with turbidity and free chlorine in two distribution systems. Am. Water Works Assoc. J. **75:**309–312.
29. **Robinson, P. J., J. T. Walter, C. W. Keevil, and J. Cole.** 1995. Reporter genes and fluorescent probes for studying the colonisation of biofilms in a drinking water supply line by enteric bacteria. FEMS Microbiol. Lett. **129:**183–188.
30. **Rozak, D. B., and R. R. Colwell.** 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. **51:**365–379.
31. **Siebel, M. A., and W. G. Characklis.** 1991. Observations of binary population biofilms. Biotechnol. Bioeng. **37:**778–789.
32. **Subra, P., M. C. Hennion, and R. Rosset.** 1989. Analyse de la matrière organique contenue dans des eaux de distribution par des méthodes chromatographiques. Analusis **17:**163–184.
33. **Szewzyk, U., W. Manz, R. Amann, K. H. Schleifer, and T. A. Stenstrom.** 1994. Growth and in situ detection of a pathogenic *Escherichia coli* in biofilms of a heterotrophic water-bacterium by use of 16S- and 23S-rRNA-directed fluorescent oligonucleotide probes. FEMS Microbiol. Ecol. **13:**169–176.
34. **van der Kooij, D., A. Visser, and W. A. M. Hijnen.** 1982. Determining the concentration of easily assimilable organic carbon in drinking water. Am. Water Works Assoc. J. **74:**540–545.
35. **van der Wende, E., W. G. Characklis, and D. B. Smith.** 1989. Biofilms and bacterial drinking water quality. Water Res. **23:**1313–1322.