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Assessment of the Bacteriological Activity Associated with Granular Activated Carbon Treatment of Drinking Water

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Bacteriological analyses were performed on the effluent from a conventional water treatment pilot plant in which granular activated carbon (GAC) had been used as the final process to assess the impact of GAC on the microbial quality of the water produced. Samples were collected twice weekly for 160 days from the effluents of six GAC columns, each of which used one of four different empty-bed contact times (7.5, 15, 30, and 60 min). The samples were analyzed for heterotrophic plate counts and total coliforms. Effluent samples were also exposed to chloramines and free chlorine for 60 min (pH 8.2, 23°C). Bacterial identifications were performed on the disinfected and nondisinfected effluents. Additional studies were conducted to assess the bacteriological activity associated with released GAC particles. The results indicated that heterotrophic plate counts in the effluents from all columns increased to 10^5 CFU/ml within 5 days and subsequently stabilized at 10^4 CFU/ml. The heterotrophic plate counts did not differ at different empty-bed contact times. Coliforms (identified as *Enterobacter* spp.) were recovered from the nondisinfected effluent on only two occasions. The disinfection results indicated that 1.5 mg of chloramines per liter inactivated approximately 50% more bacteria than did 1.0 mg of free chlorine per liter after 1 h of contact time. Chloramines and chlorine selected for the development of different bacterial species—*Pseudomonas* spp. and *Flavobacterium* spp., respectively. Carbon particles were recovered in the effluent at levels of 10 to 62 particles per liter. Scanning electron microscopy revealed that 8% of the particles had no detectable colonized bacteria, 77% were colonized with 1 to 50 bacterial cells, and 8% were colonized with several hundred to several thousand bacterial cells. On one occasion, a fecal coliform identified as *Klebsiella pneumoniae* was desorbed from a carbon particle. Disinfection studies indicated that bacteria attached to the GAC particles were extremely resistant to either chlorine or chloramines at 1.5 mg/liter, even after 40 min of exposure. These findings suggest that chloramines may be more effective than chlorine in controlling the heterotrophic plate counts resulting from GAC treatment when the bacterial population is predominantly composed of non-*Pseudomonas* spp. These studies also indicate that bacterially colonized GAC particles released into the product water are extremely resistant to disinfection.

Granular activated carbon (GAC) has been used extensively for the removal of compounds causing taste, odor, and color problems in drinking water (10, 18, 23). In addition, this treatment process is recognized as an effective means of removing organic or precursor compounds that may react with chlorine to produce trihalomethanes and other disinfection by-products (7, 18, 21). The U.S. Environmental Protection Agency recently issued a final priority list of disinfection by-products, including trihalomethanes. Maximum contaminant levels for these compounds will be issued by early 1992. Although it is not currently known what the maximum contaminant level for trihalomethanes will be, it is anticipated that it will be lowered from the present level of 100 μg/liter to either 25 or 50 μg/liter (24).

Bacterial colonization of GAC is considered to result in part from (i) the adsorptive properties of carbon, which enrich nutrient and oxygen concentrations and remove disinfectant compounds; (ii) the porous surface of the carbon particles, which provides a protective environment from fluid shear forces; (iii) the presence of a variety of functional groups on the carbon surface, which enhances microbial attachment; and (iv) neutralization of stressor compounds (15, 18, 21, 25, 27). Bacterial populations appear to develop rapidly on these carbon beds and generally follow classical sigmoidal growth kinetics (7, 15). A number of bacterial genera have been identified in GAC product water; these include *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Acinetobacter*, *Aeromonas*, *Chromobacterium*, and others. The distributions of dominant genera and their levels in effluent waters, as reported in the literature, vary significantly. This variation can probably be attributed to different source water characteristics, modes of treatment plant operation, and bacterial enumeration and identification methodologies.

Although the establishment of these organisms in carbon beds can be beneficial, GAC treatment may increase the number of bacteria entering the distribution system. The potential impact of these attached organisms in the distribution system has only recently been recognized (9, 14, 16).

To evaluate the bacteriological and disinfection problems that may be associated with GAC treatment, we performed a comprehensive pilot-scale study to determine the impact of free and attached bacteria released into the product water. Samples were collected and analyzed for (i) the release of coliforms and heterotrophic plate count (HPC) bacteria, (ii) the impact of empty-bed contact time (EBCT) on bacterial release, and (iii) the survival and identity of bacteria following disinfection with chlorine and chloramines.

MATERIALS AND METHODS

Description of the pilot plant. The GAC pilot plant was constructed entirely of stainless steel, glass, and Teflon to avoid organic contamination. The pilot plant was designed to

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receive (i) State project water, imported from the San Joaquin-Sacramento River Delta in Northern California, (ii) Colorado River water, (iii) or any desired blend of these two source waters. The pilot plant used conventional water treatment unit processes, including predisinfection of plant influent water with chlorine dioxide, rapid mixing, flocculation, sedimentation, and dual-medium filtration. The chlorine dioxide dosage ranged from 0.3 to 1.0 mg/liter, depending on the demand characteristics of the influent water, to achieve a residual of 0.04 to 0.1 mg/liter in the filter effluent. Carbon adsorption was incorporated into the pilot plant as the final step of the treatment train. The plant's six glass GAC adsorption columns (15 cm in diameter and 3 m high) used Filtrasorb 400 activated carbon (Calgon Carbon Corp., Pittsburgh, Pa.) at depths of 0.15, 0.30 (three replicate columns), 0.60, and 1.2 m (or ca. 0.5, 1, 2, and 4 ft, respectively). The respective EBCTs for these columns were 7.5, 15, 30, and 60 min. With the exception of column 3 (which used carbon particles sized at 20/50 mesh), all columns used carbon particles sized at 12/40 mesh.

Sample collection. Bacteriological samples were collected from the pilot plant influent, from the filter effluent, and from each of the GAC column effluents. Three samples (designated A, B, and C) were collected from each of the six GAC columns. With the exception of samples B and C, the samples were collected in sterile 150-ml Nalgene plastic bottles (Nalge/Sybron Corp., Rochester, N.Y.). Samples B and C were collected in sterile 1-liter Wheaton bottles (Wheaton Industries, Millville, N.J.). Following collection, all samples were transported to the laboratory on ice and processed within 4 h.

Coliform and HPC analyses. The samples were analyzed for total coliforms and HPCs by the procedures described in *Standard Methods for the Examination of Water and Wastewater* (1). In consideration of the high levels of turbidity and background HPCs, the pilot plant influent samples and column effluent sample A were analyzed for coliforms by the most-probable-number method. The filter effluent samples and disinfected column effluent samples B and C were analyzed for coliforms by the membrane filtration procedure with m-Endo agar LES (Difco Laboratories, Detroit, Mich.). HPC analysis was performed by membrane filtration with R2A medium (Difco) (m-R2A method) and incubation for 7 days at 28°C before enumeration.

Disinfection of column effluents. Chlorine and chloramine disinfection studies were performed on column effluent samples B and C. Sample A was the untreated control. Sample B was exposed to 1.5 mg of chloramines per liter (by preammoniation, at a 3:1 ratio of chlorine to nitrogen) for 1 h (pH 8.2, 23°C). Sample C was exposed to 1.0 mg of free chlorine per liter under the same conditions. The final residuals were approximately 0.75 and 1.3 mg/liter for chlorine and chloramines, respectively.

Identification of microbial isolates. Bacterial isolates from the plant influent, filter effluent, and column effluent samples were collected and identified after 8 and 54 days of pilot plant operation. Column 2 (15-min EBCT) and column 6 (60-min EBCT) were selected to represent the range of likely EBCT conditions for a full-scale treatment facility.

A total of 36 to 44 colonies were randomly selected from the R2A plates for each site. Selected colonies were streaked onto R2A plates to determine purity. Following preliminary classification by Gram staining, isolates were identified by three methods. Gram-negative, nonfermentative bacteria were identified by the API NFT diagnostic system (Analytab Products, Plainview, N.Y.). Organisms not identifiable by the NFT system were identified by the method of Ward et al. (26). All gram-positive bacteria were classified as described in *Bergey's Manual of Systematic Bacteriology* (11).

Recovery and characterization of released GAC particles. Approximately 1,000 liters of column 4 effluent was continuously collected in a sterile 2-liter flask and pumped from this flask at a rate of approximately 160 ml/min through two sterile 10-μm-pore-size membrane filters (Nuclepore Corp., Pleasanton, Calif.) run in parallel. The filters were removed and aseptically placed on Whatman no. 1 sterile filter paper (Whatman, Inc., Clifton, N.J.), and the carbon particles were enumerated under 40× magnification with a stereoscope (Reichert Stereo Star Zoom; Cambridge Instruments, Buffalo, N.Y.).

Particle size analysis. For particle size determination, the membranes were cut in half and examined with a light microscope (model BH2; Olympus Corp., Tokyo, Japan) under 400× magnification. The diameters of at least 30 randomly selected particles were measured.

SEM analysis of colonized particles. Scanning electron microscope (SEM) analysis of carbon particles collected from the effluent was performed to determine the degree of bacterial colonization. The 10-μm-pore-size Nuclepore filters were cut into quarters and placed on a sterile 0.22-μm-pore-size Nuclepore filter contained in a membrane filtration apparatus (Millipore Corp., Bedford, Mass.). Cold 2.5% glutaraldehyde solution (15 ml in 0.1 M sodium cacodylate buffer; pH 7.2) was placed over the Nuclepore membranes for 1 h at room temperature and slowly removed by vacuum filtration, and the membranes were rinsed three times with 10 ml of cacodylate each time. The particles were dehydrated with 10-ml volumes of reagent-grade acetone at increasing concentrations of 10, 30, 50, 70, 90, and 100% for 5 min at each concentration. The Nuclepore membranes were rinsed twice with 100% acetone, air dried, cut, and placed on an SEM specimen mount (Ted Pella, Inc., Tustin, Calif.). The particles were coated with gold (approximately 300 Å [30 nm]) at 15 mA in a sputter coater (Hummer V; Technics, Inc., Alexandria, Va.) and examined with an SEM (model 1000A; AMRAY, Inc., Bedford, Mass.). Approximately 100 particles were randomly selected and scored for size and degree of bacterial colonization.

Desorption of bacteria attached to released GAC particles. The removal of bacteria attached to GAC particles was accomplished by a modification of the procedure of Camper et al. (8). The complete membrane was placed in a sterile 50-ml centrifuge tube containing 25 ml of phosphate buffer (1), vortexed for approximately 5 min, and sonicated for 5 min in a Bransonic 32 sonicator (Branson Sonic Power Co., Danbury, Conn.). The membrane was removed and reexamined under the stereoscope to determine the efficiency of particle removal. Vortexing and sonication resulted in the removal of approximately 85% of the carbon particles from the membrane. The membrane was aseptically removed from the 50-ml centrifuge tube and discarded. Unattached HPC bacteria in this solution were inactivated by the addition of chlorine (final concentration, 2 mg/liter; pH 7.2) (8). This suspension was gently mixed for 20 min at room temperature (23°C), after which a 1-ml portion was removed, added to a phosphate buffer tube containing sodium thiosulfate, and examined by the m-R2A method for any surviving unattached bacteria. To enumerate the particle-attached bacteria, we placed the contents (carbon particles) of the 50-ml centrifuge tube into a Waring blender (model 34BL22; Dynamics Corp. of America, New Hartford, Conn.) already containing 25 ml of iced, double-strength desorption solution

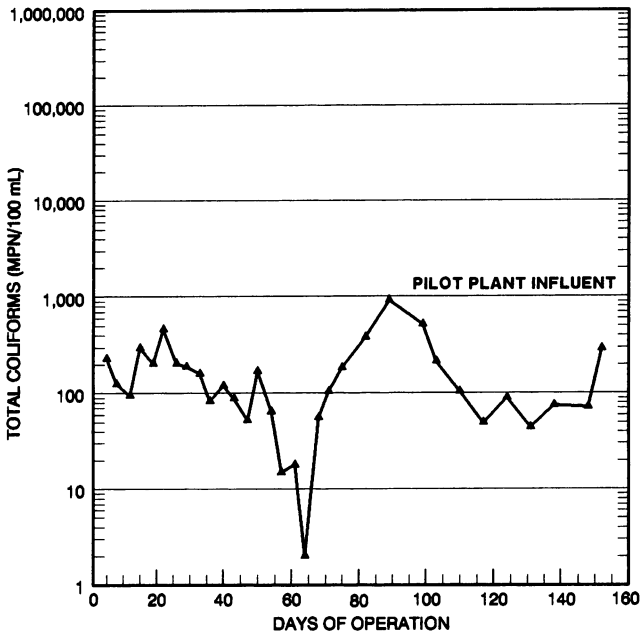

FIG. 1. Total coliform levels in plant influent during pilot plant studies. MPN, Most probable number.

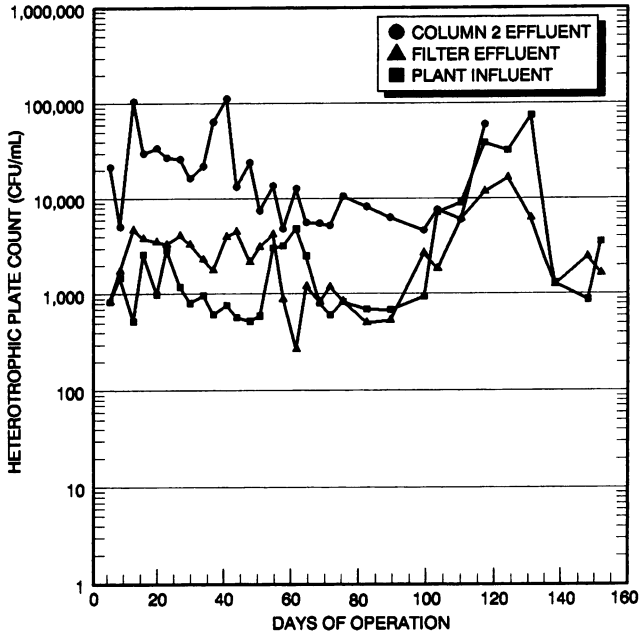

FIG. 2. HPC levels in pilot plant influent, filter effluent, and GAC column 2 effluent.

(single-strength desorption solution contains 10^{-6} M Zwittergent 3-12 [Calbiochem-Behring Corp., La Jolla, Calif.], 10^{-3} M EGTA [Sigma Chemical Co., St. Louis, Mo.], and 0.01% Bacto-Peptone [Difco] in a buffered solution of 0.01 M Tris [Sigma] at pH 7.0). The solution was blended for 1 min on high (final temperature, <23°C), removed, and enumerated for desorbed bacteria. Detached coliforms were analyzed by mixing the entire 50-ml desorption solution with an equal volume of phosphate buffer (1) and using a 10-tube most-probable-number method.

Inactivation of bacteria attached to GAC by chlorine and chloramines. Carbon was removed from the top layer of column 4 and divided into three 0.5-g (wet-weight) portions. Each portion was resuspended in 20 ml of phosphate buffer (1), and the suspension was mixed gently for approximately 30 min at room temperature to dissociate loosely attached bacteria. The levels of unattached bacteria were quantified by the m-R2A method described above. The number of attached organisms in tube 1 was determined by subtracting the number of unattached bacteria from the number of bacteria obtained after desorption. Tubes 2 and 3 were exposed to chlorine and chloramines, respectively (final concentration, 1.5 mg/liter; pH 7.0) and gently agitated for 40 min at 23°C. The disinfectants were neutralized with sodium thiosulfate and analyzed to determine the surviving unattached HPC and coliform bacteria by the above-described procedures. Desorbed bacteria from untreated, chlorine-treated, and chloramine-treated carbon were isolated and identified as described above.

RESULTS

Coliform analyses. The levels of total coliform bacteria in the pilot plant influent remained generally constant, with a geometric mean of approximately 100 coliforms/100 ml (Fig. 1). A notable increase in coliform counts was observed following day 65. This occurrence may have been caused by the sudden increase in the use of State project water (0 to 85% blend) during this period. Total coliforms at levels of 1 and 2 coliforms/100 ml were recovered on only two occasions from the filter effluent and were detected on only three occasions in the column effluent. After 17 days of pilot plant operation, 2 coliforms/100 ml were recovered from the column 1 effluent. In the second episode (occurring after 21 days of pilot plant operation), 5 coliforms/100 ml were recovered from both columns 3 and 6 and 2 coliforms/100 ml were recovered from column 5. In the last episode (occurring after 42 days of pilot plant operation), 5 coliforms/100 ml were recovered from column 5. In all three occurrences, the coliforms were identified as *Enterobacter* spp. No coliforms were detected in the column effluents following disinfection with either chlorine or chloramines.

HPC analyses. Figure 2 shows HPC levels in the pilot plant influent, filter effluent, and column 2 effluent (nondisinfected). The geometric means for HPC levels in the plant influent and filter effluent during the first 70 days of pilot plant operation were approximately 1,100 and 2,500 CFU/ml, respectively. HPC levels were consistently higher in the filter effluent than in the plant influent until approximately day 100, when the chlorine dioxide residual in the filter effluent was increased from 0.04 to 0.1 mg/liter. This increase resulted in a reduction of HPC levels in the filter effluent. Beginning on approximately day 90, bacterial levels in the plant influent and filter effluent increased considerably, peaking around day 120 at 10^4 CFU/ml and returning to initiate levels of 10^3 CFU/ml by day 130. This change was believed to have resulted from a pilot plant increase in the use of state project water, which typically contains higher concentrations of HPC bacteria than does Colorado River water. There was also a notable HPC increase in the plant influent on day 56 which may have been caused by an increase in the blend of state project water from 0 to 85%. In general, HPC levels increased throughout the treatment train (Fig. 2).

The HPC levels in the GAC column effluents were similar

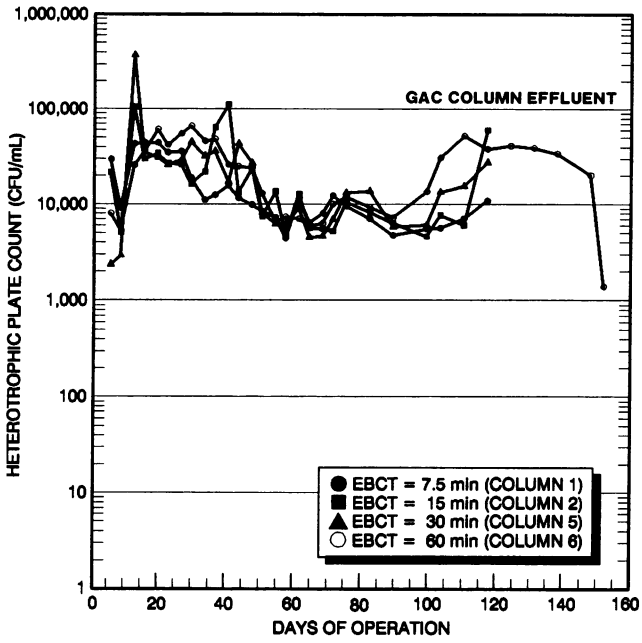

FIG. 3. HPC levels in GAC column effluents for various EBCTs (7.5, 15, 30, and 60 min).

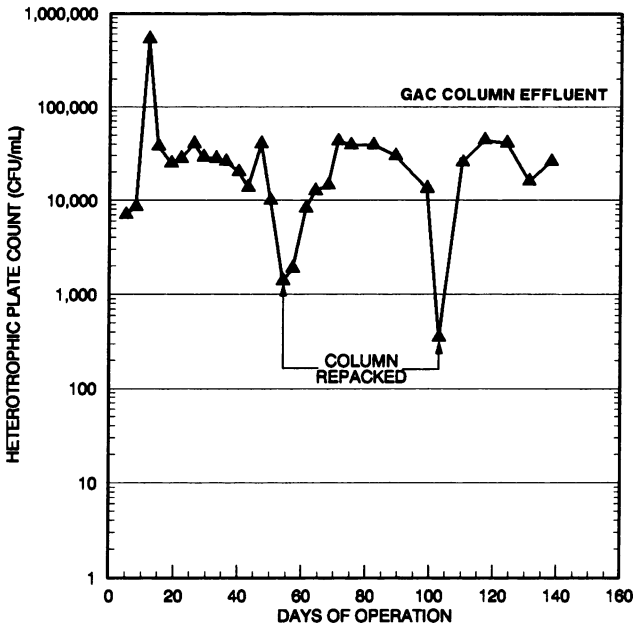

FIG. 4. Colonization kinetics of HPC bacteria following repacking of GAC in column 4 on days 51 and 100.

for all EBCTs (Fig. 3). Statistical analyses with a two-way analysis-of-variance statistical program (RS/1, version 3.0; BBN Software Products Co., Cambridge, Mass.) on a minicomputer (VAX 8200; Digital Equipment Corp., Maynard, Mass.) confirmed that the HPC levels in these column effluents were not significantly different ($\alpha = 0.05$). For all column effluents, the HPC levels increased dramatically from days 3 to 7 (e.g., 10^3 to 10^6 CFU/ml) of operation and then declined rapidly between days 7 and 10 to a constant level of approximately 10^4 CFU/ml (Fig. 3). Similar colonization kinetics were observed following column repacking (Fig. 4). On days 51 and 100, when column 4 was repacked, HPC levels returned to previous levels after a short regrowth phase.

Chlorine and chloramine disinfection of GAC column effluents. Effluent samples from column 1 produced approximately 0.5 to 1.0 $\log_{10}$-lower HPC levels when exposed to 1.5 mg of chloramines per liter than when exposed to 1.0 mg of chlorine per liter under the same conditions (Fig. 5). This relationship was similar for each of the columns (Fig. 6). No coliforms were recovered after exposure to either chlorine or chloramines. This relationship was consistent throughout the monitoring period for each EBCT.

Bacterial identifications. The predominant genera appearing in the plant influent, filter effluent, and column effluent (nondisinfected control) were *Pseudomonas* and *Flavobacterium* (Table 1). Additional genera recovered from these sites included *Empedobacter*, *Alcaligenes*, *Acinetobacter*, *Achromobacter*, and *Moraxella*. There was no apparent relationship between EBCT and bacterial diversity.

There appeared to be a decrease in bacterial diversity from plant influent to filter effluent. However, the bacterial genera in the column effluent were similar to those in the filter effluent. Column effluent samples exposed to chloramines developed bacterial populations that were exclusively white-pigmented *Pseudomonas* spp. By contrast, bacterial genera surviving exposure to chlorine were predominantly red-pigmented organisms identified as *Pseudomonas* and *Flavobacterium* spp. The *Pseudomonas* species surviving chlorination were different from those surviving chloramination. *Pseudomonas* spp. and *Pseudomonas diminuta* were the only species identified from the chloraminated samples, whereas *Pseudomonas* spp., *Pseudomonas vesicularis*, *Pseudomonas* (*Xanthomonas*)-like spp. (26), and a mixed group of red-pigmented *Pseudomonas* spp. and *Flavobacterium* spp. were recovered from the chloraminated samples (Table 2). The organisms in this mixed group were variable

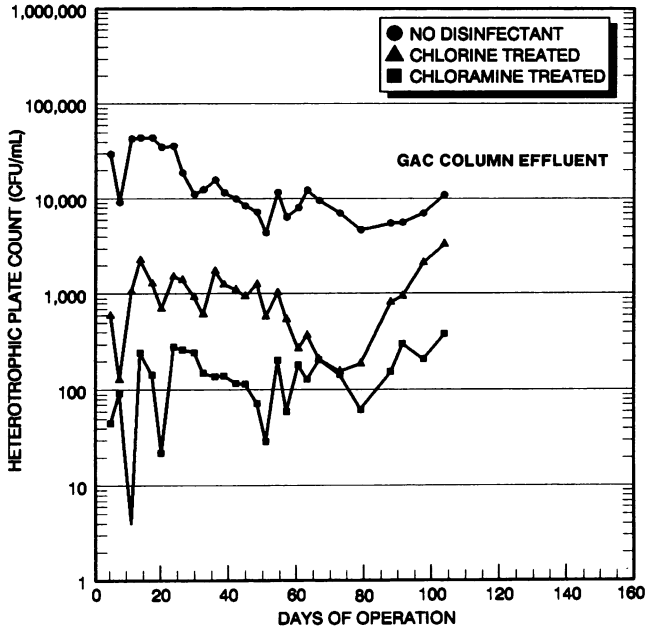

FIG. 5. HPC levels in GAC column 1 effluent following exposure to chlorine (1.0 mg/liter; pH 8.0) and chloramines (1.5 mg/liter; pH 8.0) for 1 h.

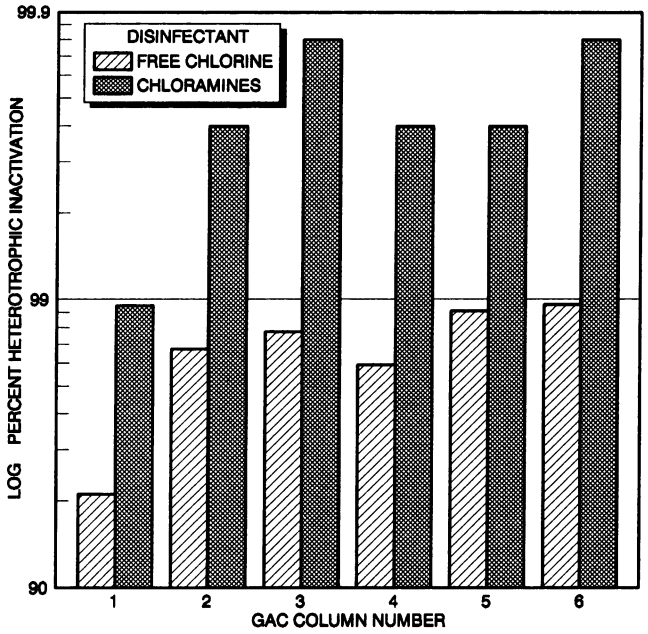

FIG. 6. HPC levels in all GAC column effluents following exposure to chlorine (1.0 mg/liter; pH 8.0) and chloramines (1.5 mg/liter; pH 8.0) for 1 h.

for motility. Because of the limitations of currently available identification schemes, it was difficult to classify these organisms definitively as either *Pseudomonas* or *Flavobacterium* spp.

Bacterial colonization of effluent GAC particles. Approximately 36 carbon particles (standard deviation, 17; range 10 to 62) were released per liter of column 4 effluent. However, the actual number may have been much higher, as a 10-μm-pore-size filter was used to collect the released carbon particles. This membrane size was selected to collect particles large enough to have significant numbers of colonized bacteria. The particles ranged in size from 2 to 40 μm in diameter, with a mean diameter of 5.4 μm (standard deviation, 4.6). (The actual mean may have been lower, as a 10-μm-pore-size filter was used.) The number of viable bacteria attached to each particle, as determined by the m-R2A method, ranged from 0 to approximately 7, with a mean of 3 (standard deviation, 3). On the basis of an average release of 36 particles per liter, it is conservatively estimated that between 0 and 434 viable attached bacteria (mean, 108; standard deviation, 54) were released per liter of column 4 effluent.

SEM analysis indicated that 8% of the particles had no detectable colonized bacterial cells (Fig. 7), 77% were colonized with 1 to 50 bacterial cells, 7% were colonized with 51 to 100 bacterial cells, and 8% were covered with several hundred to several thousand or more bacterial cells. Because of the asymmetrical distribution of colonized particles and the inability of SEM to determine viability, the actual mean level of viable bacteria per particle could not be determined.

Disinfection resistance of bacterially colonized GAC. Nei-

TABLE 2. Identification of HPC bacterial species in GAC column 2 effluent following chlorination or chloramination[a]

Organism	% of isolates following:	
	Chlorination[b]	Chloramination[c]
Pseudomonas spp.	5	34
Pseudomonas diminuta	0	53
Pseudomonas vesicularis	11	4
Pseudomonas (*Xanthomonas*)-like spp.	26	8
Pseudomonas or *Flavobacterium*	37	0
Flavobacterium cytophyga	15	0
Unidentified	4	0
Total number of isolates	73	73

[a] Composite of 8 and 54 days of pilot plant operation.
[b] 1.0 mg/liter, pH 8.2, 60 min.
[c] 1.5 mg/liter, pH 8.2, 60 min.

TABLE 1. Identification of HPC bacteria in the GAC study[a]

Genus	% of isolates in:							
	Plant influent	Filter effluent	GAC effluent					
			Column 2[b]			Column 6[c]		
			Control	NH_2Cl[d]	HOCl[e]	Control	NH_2Cl[d]	HOCl[e]
Pseudomonas	63	75	88	100[f]	42[g]	71	100[f]	28[f]
Pseudomonas or *Flavobacterium*		17	4		37[g]			47[g]
Flavobacterium	11	1	1		15[g]			13[g]
Empedobacter	3							
Alcaligenes	4							
Acinetobacter	1							
Achromobacter	8		1					
Moraxella	3		1			24		1
Bordetella			1					
Staphylococcus			1					
Unidentified	6	3	1		3	5		11
Total number of isolates	72	72	76	73	73	73	73	81

[a] Composite for 8 and 54 days of pilot plant operation.
[b] 15-min EBCT.
[c] 60-min EBCT.
[d] 1.5 mg/liter, pH 8.2, 60 min.
[e] 1.0 mg/liter, pH 8.2, 60 min.
[f] Predominantly white-pigmented colonies and some red-pigmented colonies.
[g] All red-pigmented colonies.

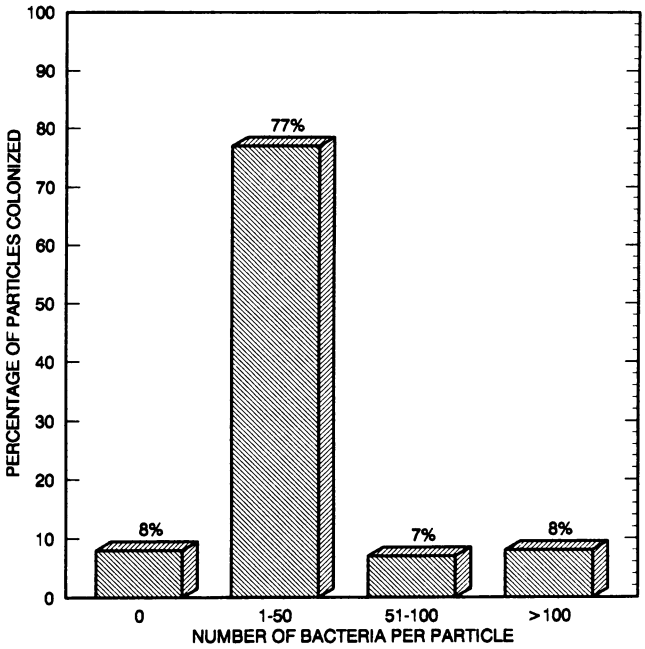

FIG. 7. Number of bacteria attached to each GAC particle as determined by SEM analysis.

ther chlorine nor chloramines (final concentration for both disinfectants, 1.5 mg/liter; pH 7.0) proved effective in inactivating bacteria attached to GAC (Fig. 8). The reduction in attached bacteria after 40 min of contact time with either disinfectant was <0.5 $\log_{10}$.

Identification of the bacteria desorbed from GAC particles exposed to chlorine or chloramines (Table 3) revealed no

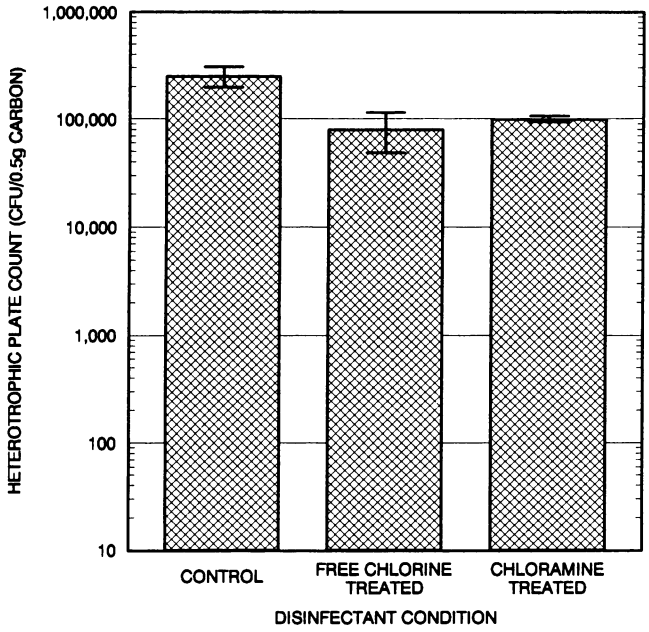

FIG. 8. Inactivation of GAC particle-bound bacteria by chlorine (1.5 mg/liter; pH 7.0) and chloramines (1.5 mg/liter; pH 7.0). Error bars, ±1 standard deviation.

TABLE 3. Identification of HPC bacteria desorbed from GAC particles

Genus	% of isolates:			
	In column effluent	Particle associated		
		Untreated[a]	NH_2Cl[b]	HOCl[b]
Achromobacter	3	0	3	6
Flavobacterium	9	3	3	0
Klebsiella	0	0	1	0
Moraxella	3	0	6	16
Pseudomonas	84	94	88	78
Unidentified	0	3	0	0
Total number of isolates	32	34	33	32

[a] Not exposed to disinfectant.
[b] Final concentration, 1.5 mg/liter; pH 7.0; 23°C; 40 min.

significant differences between the bacteria recovered from the column effluent and those attached to GAC particles. The predominant genera were *Pseudomonas* and *Flavobacterium*. Also, no appreciable differences were apparent between the genera surviving chlorine treatment and those surviving chloramine treatment. It is important to note that, on one occasion, a fecal coliform identified as *Klebsiella pneumoniae* was recovered from carbon particles exposed to chloramines.

DISCUSSION

The use of GAC for water treatment may increase significantly in the United States as water utilities strive to meet regulations governing the types and concentrations of disinfection by-products released into drinking water supplies. Although GAC has been found to be generally effective for the removal of disinfection by-products and compounds causing objectionable taste and odor (10, 17, 18, 21, 23), it can have an adverse impact on the bacteriological quality of the water in the distribution system. The goal of this study was to examine several bacteriological effects of GAC in treated water, including (i) the release of coliforms, (ii) the release of HPC bacteria, (iii) the impact of EBCT on the release of particles and bacteria, and (iv) the bacteriological activity associated with colonized GAC particles.

In this study, the use of GAC did not lead to an increase in the coliform level in the treated water effluent as compared with the plant influent coliform level. There was only a sporadic increase in coliforms in the column effluent as compared with those in the filter effluent. Low coliform levels were observed on two occasions in the filter effluent, in contrast to three occasions on which coliforms were recovered from the column effluent during 160 days of pilot plant operation. Furthermore, no coliforms were recovered from the column effluent following disinfection with either chlorine or chloramines.

In contrast to our findings, other investigators have reported the release of substantial numbers of coliforms in water treated with GAC (5, 16, 28). The differences between our results and those of others may in part have resulted from the fact that the plant influent water in the present study was disinfected with chlorine dioxide prior to GAC treatment. This step may have limited the amount of coliforms potentially colonizing the GAC columns. In support of this contention, no coliforms were detected in the filter effluent, with the exception of two occasions on which 1 and

2 coliforms were recovered after 42 and 151 days of pilot plant operation, respectively.

The HPC bacterial growth associated with GAC treatment was considerable, as has been documented by others (2, 9, 15, 19). In general, HPC levels increased throughout the treatment train until post-GAC disinfection. We found that the HPC colonization and release kinetics for all the EBCTs were similar: HPC levels increased dramatically from days 3 to 7 (e.g., 10^3 to 10^6 CFU/ml) of operation and then declined rapidly between days 7 and 10 to a constant level of approximately 10^4 CFU/ml. Similar patterns have been reported by other researchers (3, 7, 9, 15, 21).

An interesting finding of this study was that chloramines were more effective than chlorine in reducing HPC levels. The application of chlorine (1.0 mg/liter) and chloramines (1.5 mg/liter) for 1 h to GAC effluents reduced HPC levels by 99 and 99.9%, respectively (Fig. 6). Although chlorine is generally considered a much more effective bactericide than are chloramines, work by Wolfe et al. (29) showed comparable amounts of HPC inactivation in a finished water reservoir exposed to chlorine and chloramines. This result was attributed to the different resistance patterns of the various bacterial genera. The organisms surviving chlorine exposure were exclusively red-pigmented bacteria identified as *Flavobacterium* spp., whereas the organisms surviving chloramine exposure included predominantly nonpigmented species. This pattern was also observed in the present study. The chlorine-resistant organisms were red-pigmented bacteria identified as a mixture of *Pseudomonas* and *Flavobacterium* spp. In contrast, the chloramine-resistant organisms were almost exclusively white-pigmented *Pseudomonas* spp. This difference may explain why chloramine exposure in this study resulted in lower HPC levels than did chlorine exposure.

The predominant genera appearing in the plant influent, filter effluent, and column effluent were *Pseudomonas* and *Flavobacterium*. The genera *Achromobacter*, *Moraxella*, *Acinetobacter*, *Empedobacter*, and *Alcaligenes* were observed to a lesser extent. All of these genera are common aquatic organisms and have been frequently recovered by other investigators conducting GAC studies (6, 15, 19, 28). The filter effluent, however, appeared to have less bacterial diversity than did the plant effluent, suggesting that some selection occurred as a result of the treatment conditions.

An important part of this study was the evaluation of the bacteriological impact of released GAC particles. Direct observation by SEM analysis of GAC particles released in the effluent indicated that 85% were colonized with less than 50 bacterial cells but that 8% had several hundred to several thousand bacterial cells. Ridgway and Olson (20), in a study based on SEM analysis of particles appearing in the distribution system, observed that 17% of the particles were colonized with 10 to 100 bacterial cells. These researchers indicated that even if only 17% of all distribution system particles were colonized with 10 to 100 bacteria per particle, conventional plate count procedures would underestimate the actual bacterial count by a factor of 1,500 to 15,000 per milliliter. Therefore, even though 8% of the GAC particles observed in the present study were colonized with several hundred to several thousand bacterial cells, the release of only a few particles could introduce significant numbers of organisms into the distribution system.

Because of the potential for particle colonization, conventional bacterial recovery techniques could greatly underestimate the number of released organisms. Camper et al. (9), using standard coliform recovery methods, found that the actual coliform levels in GAC effluent were underestimated by a factor of 122 to 1,194.

Many investigators have commented on the potential health impacts of bacteria or their metabolic products released from GAC treatment (5, 7, 16, 18, 25). In a study reported by Camper et al. (9) involving GAC effluent samples collected from water utilities with operational GAC contactors, 40% of the samples contained bacterially colonized GAC particles. More importantly, 17% of these samples had attached coliforms, 28% of which tested positive as fecal coliforms. GAC particles may also act as a nutrient source to stimulate the growth of autochthonous bacterial populations in the distribution system (21, 25).

Our study determined that attached organisms were not significantly inactivated by either chlorine or chloramines. Some researchers have suggested that chloramines are a superior bactericide for attached organisms (13), whereas others have stated that chlorine is more effective for inactivating particle-attached bacteria (4). These disparate observations may be attributable to differences in the disinfectant resistance characteristics of the predominant bacterial genera or to some interaction between the disinfectant and the chemicals on the particle surface. LeChevallier et al. (14) indicated that bacteria attached to carbon particles were resistant to 2.0 mg of free chlorine per liter for up to 1 h and, in a further study (13), demonstrated that bacteria (including pathogenic *Salmonella* and *Shigella* spp.) attached to GAC particles were much more resistant than were unattached bacteria to inactivation by chlorine and chloramines. However, they noted that chloramines inactivated more GAC-associated bacteria than did chlorine and posited that this difference was a result of greater penetration by chloramines than by chlorine. Although attached organisms are thought to be resistant because of the presence of extensive polymer layers (22), LeChevallier et al. (12, 14) demonstrated that GAC-attached, polymer-producing organisms were comparable to non-polymer-producing organisms in their resistance to chlorine. It was concluded that protection may result from the neutralizing action of the carbon on the disinfectant and/or the inability of the disinfectant to come into contact with organisms.

In summary, the results of the present study indicate that (i) GAC was readily colonized by bacteria, (ii) these bacteria were similar to those recovered from the filter effluent, (iii) chloramines were more effective in reducing levels of HPCs in GAC effluent than was chlorine, (iv) 36 GAC particles with a mean diameter of 5.4 μm were released per liter, and (v) the bacteria on the GAC particles that were released were highly resistant to chorine and chloramines.

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