76. E. C. Nieminski and J. E. Ongerth, *J. AWWA* **87**(9), 96–106 (1995).
77. J. Swertfeger et al., *J. AWWA* **91**, 90–100 (1999).
78. L. J. Robertson et al., *U.K. Water Res.* **34**, 2310–2322 (2000).
79. A. Garcia et al., *Proc. Water Environment Federation Technical Conference*, 1999.
80. T. Hirata and A. Hashimoto, *Proc. Int. Symp. Waterborne Cryptosporidium*, AWWA, Denver, Col., 1997, pp. 183–193.

GRANULAR ACTIVATED CARBON, BACTERIOLOGY OF

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The goal of drinking water treatment has always been to provide microbiologically safe water to the consumer. To accomplish this, especially when surface water is being treated, gravity filters containing sand and/or anthracite are typically used to remove particulate matter. When filtration is followed by disinfection, pathogens should be removed and the microbial load of the water reduced to an acceptable level. As water quality regulations have become more restrictive, granular activated carbon (GAC) has been used to a greater extent as a filter medium because of its ability to adsorb regulated synthetic organic carbon compounds, materials that contribute to taste and odor in finished water, and organic matter that leads to the formation of disinfection by-products. GAC is known to become colonized with bacteria, and this trend has been exploited in treatment by using the organisms on biologically active carbon (BAC) to metabolize a fraction of the organic carbon found in the water. However, there has been concern that indicator organisms and bacteria of public health interests may colonize and proliferate on GAC and BAC filters. For these bacteria to be found in finished water they must successfully colonize and compete with the indigenous organisms (or be retained in large numbers), be released from the filter and penetrate the disinfection barrier. This review describes these issues and the beneficial aspects of BAC filtration in water treatment.

PROPERTIES OF GAC

Granular activated carbon (GAC) has the beneficial property of adsorbing compounds from water. Consequently, its use in drinking water treatment has increased substantially over the past few decades due in large measure to increasingly stringent regulations on finished water quality. Of rising importance are regulations related to the requirement to remove organic compounds involved in the formation of disinfection by-products. GAC can also adsorb gasoline constituents, pesticides, and other regulated synthetic organic compounds. The aesthetic properties of potable water can also be improved because GAC can remove a variety of organic compounds that cause tastes, odors, and color.

The adsorptive properties of GAC are related in part to its surface area. GAC particles are characterized by very high surface area ranging from a few hundred to 1,500 m^2/g, most of which is provided by their porous nature (1). Pore size distributions are in the range of $1–10^4$ Å, with most pores being smaller than the average bacterium (1–2 μm or $1–2 \times 10^4$ Å in length). Although the majority of the pores is small, the porosity of GAC appears to be an important factor that enhances the ability for bacteria to colonize GAC particles in water treatment systems.

GAC can also react with oxidizing disinfectants such as free chlorine (1,2), combined chlorine (3) and chlorine dioxide (4). This property may also be important in providing a hospitable environment for bacteria attached to GAC in filters subjected to chlorinated backwash water.

COLONIZATION OF GAC

Virtually any surface immersed in water will eventually become colonized with microorganisms, and granular activated carbon is no exception. Indirect evidence for colonization was noted by treatment system operators who observed that microbial counts emanating from GAC filters frequently exceeded those found in the influent. This was also observed in early pilot scale work with a GAC filter, and the increase in counts was attributed to the growth and subsequent release of the organisms from biofilms on the filter media into the effluent water (6). This phenomenon has led to the belief in some industries that GAC columns act as "bug farms" and are therefore undesirable in the treatment train (7). Biological activity on activated carbon in the drinking water industry was initially investigated in the 1970s (8–10), with a subsequent flurry of research on the properties of GAC that make it conducive to bacterial colonization.

One such property is the strong affinity between GAC and bacterial cells. In experiments where the initial attachment of *Escherichia coli* to fresh powdered activated carbon was followed, nearly 100% of the initially suspended bacteria attached within 30 minutes (11). In similar experiments, approximately 90% attachment was seen for *Klebsiella oxytoca* (12) and *E. coli*, (13) on fresh GAC. Rollinger and Dott (14) demonstrated nearly 100% attachment of *E. coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa* within six hours, whereas *Pseudomonas putida* required four days.

Another property that may enhance bacterial colonization is the adsorption of organics in the pores of the carbon. These organics may then act as a growth substrate for the attached bacteria. Davies and McFeters (12) reported that the growth rate of attached *Klebsiella oxytoca* was 10 times higher than suspended cells in a low nutrient medium containing glutamate as the carbon source. The GAC had been exposed to the glutamate before colonization by the bacteria. The attached organisms were also more active, as determined by tritiated thymidine and uridine uptake. Compared with the suspended cells, the attached bacteria did not exhibit a reduction in cell size, that is typical for organisms entering into a starvation

state. Others have noted that organic carbon adsorbed on GAC can desorb, be transported out of the micropores, and then be degraded by bacteria attached to GAC (15,16). This mechanism may enhance the attachment of bacteria and subsequent growth of biofilms on carbon particles.

Regardless of the mechanisms, it is common knowledge that bacteria populate GAC in water-treatment systems. The actual numbers will vary depending on location, time of year, and the enumeration technique used. At one location, Parsons (17) found values ranging from 1.5×10^4 to 1.8×10^8 colony-forming units (CFU) per gram wet weight. The high end of this range was comparable to the average value of 3.6×10^8 CFU/g wet weight carbon at a New Jersey pilot plant (18). In another pilot system, culturable cell counts varied from 3×10^5 to 6×10^7 per gram (dry weight) of carbon (6). These same authors used ATP to estimate cell concentrations, and obtained levels of 5 to 490 ng ATP per gram dry weight of GAC. Using C^{14} glucose uptake to assess biomass concentrations on carbon, Prevost et al. (19) reported a range of approximately 15 μg C/cm^3 to 7.5 μg C/cm^3, depending on the location within the filter. If the biomass is assessed using a phospholipid assay, concentrations varied in an experimental carbon filter system from 500 to 20 nmols lipid-P/g (20).

Scanning electron microscopy images of GAC particles from drinking water filters have shown that the surfaces are colonized by a diverse suite of morphological types, including filaments, rods, cocci, fungi, and protozoans (21–23). A recently taken image of a particle from a laboratory biologically active carbon column is shown in Figure 1. These organisms are often associated with the macropores and cracks on the surface of the carbon (13) where they are most likely protected from the mechanical effects of shear and scraping from backwashing of the filters. Images of these particles also indicated that the organisms had produced extracellular polymeric substances (EPS, see Fig. 2), suggesting that the organisms were actively attaching and growing on the surfaces.

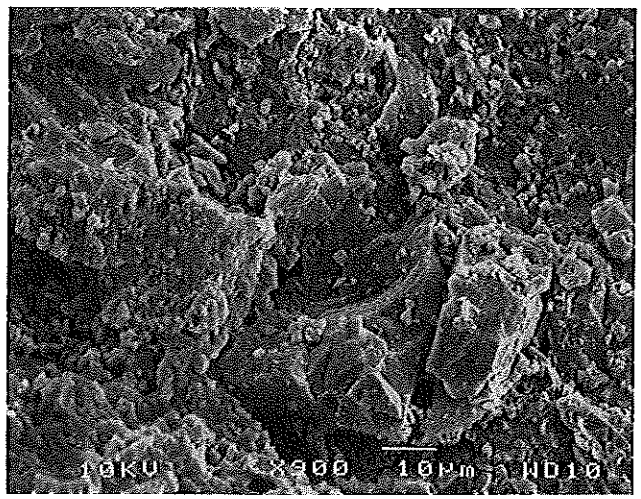

Figure 1. Scanning electron micrograph of microorganisms and detritus associated with the surface of granular activated carbon.

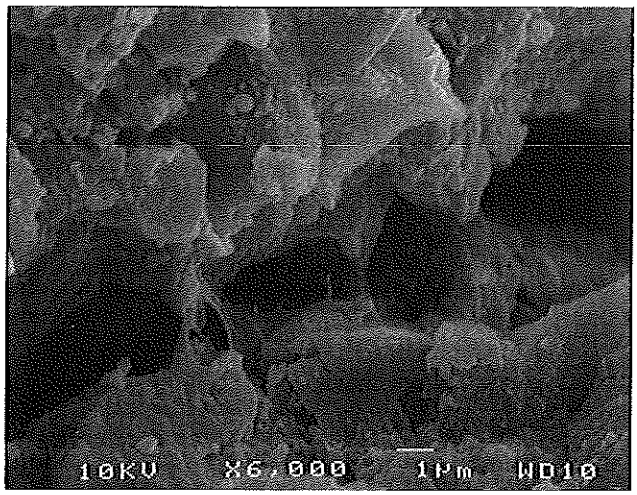

Figure 2. Scanning electron micrograph illustrating the presence of extracellular polymeric substances (EPS) in the crack of a granular activated carbon particle.

The bacteria colonizing and proliferating on the surface of GAC are likely to be nonpathogenic heterotrophs and autotrophs because the carbon and energy sources present in water support the growth of these types of organisms. Bacteria typically identified in GAC filters and their effluents are common soil and water organisms. Using culturing techniques and metabolic characteristics for classification, researchers have identified *Achromobacter*, *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Corynebacterium*, *Pseudomonas*, and *Moraxella* (14,17,22,24). At one plant, members of the genera *Pseudomonas*, *Acinetobacter*, *Bacillus*, and *Corynebacterium* were found on the filter media, with effluent heterotrophic plate counts ranging from 0 to 600 CFU/ml (25). Wilcox and associates (6) detected *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Micrococcus*, *Microcyclus*, *Paracoccus*, and *Pseudomonas* in water and on GAC particles from a pilot facility. At other locations, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Aeromonas*, *Achromobacter*, *Chromobacterium*, *Corynebacterium*, *Arthrobacter*, *Caulobacter*, *Nitrosomonas*, *Nitrobacter*, and actinomycetes were isolated from GAC filter media (26,27). Using fatty acid analysis for bacterial identification, Norton and LeChevallier (28) found 19 gram-negative and 5 gram-positive genera in biofilms on GAC. Gram-negative bacteria predominated, with the highest number of isolates belonging to the genera *Acidovorax*, *Hydrogenophaga*, *Pseudomonas*, *Xanthomonas*, and *Sphingomonas*. The majority of these isolates matched the organisms identified in the filter effluent.

It is also possible for coliforms to grow on GAC filter media. The proliferation and subsequent release of these organisms may cause a violation of the total coliform rule (TCR), even though there is no immediate fecal contamination. The TCR uses the presence of coliforms to indicate the potential presence of enteric bacterial pathogens in drinking water. Requirements state that

fewer than 5% of water samples collected on a monthly basis from the distribution system can be positive for total coliforms. The potential for positive tests resulting from detached biofilm coliforms is obviously of great concern to utility operators. There are several cases where coliforms (*Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia, Hafnia, Aeromonas*) have been found in filter effluent when they were absent in the influent water (6,24,29,30,31), suggesting that the organisms were accumulating and replicating in the filter. This is supported by the observation that coliforms such as *Citrobacter freundii, Enterobacter cloacae, Klebsiella pneumoniae,* and *Escherichia coli* have been detected in filter media samples (13,32). The growth of coliforms in filter media may also contribute to the observations of Bucklin and coworkers (33) who reported elevated coliform levels in filter effluent the first few hours after backwash. It is important to note that coliforms are not typically isolated from GAC filters, even if detailed analyses are performed (14,22,28), indicating that colonization may be a site-specific issue.

BIOLOGICAL FILTRATION

Biological filtration uses filter media, usually GAC, which has been optimized for the growth of indigenous heterotrophic bacteria that degrade the available fraction of organic carbon in the water. The filter media used in the process is termed biologically active carbon (BAC). Benefits of biological filtration include improved total organic carbon (TOC) removal and reduced disinfectant demand (35–38), improved aesthetic properties (38), and the potential for reducing microbial growth in distribution systems (39).

The process was first implemented in western European countries nearly 20 years ago (10,40,41). In the most traditional form, conventional treatment including filtration to remove particles takes place upstream from the biological filters, and ozonation is often included in the treatment train. The downstream biological filters are operated with exhausted carbon, that is, the chemisorptive capacity of the GAC has been exceeded. The surfaces of the filter media act as a support for microbial attachment and growth, resulting in a biofilm adapted to using the organic matter found in that particular water. TOC removals in these filters range from 5 to 75% (42). Conversion of organic carbon to biomass can eventually lead to a complex ecosystem containing higher life forms such as oligochetes and rotifers (43). Controlling the amount of biomass by periodic backwashing is necessary so that these organisms are not released into the filtered water (44).

One of the first observations in the full-scale use of biological filtration was that the type of filter media had an influence on the net removal of organic carbon from the water. For instance, although rapid sand filters do have the capacity to biologically remove carbon (45–48), GAC filters have superior performance [(49); comparison between DeWaters and DiGiano, (50) and Hozalski and coworkers, (51)]. LeChevallier and coworkers (49) demonstrated that there were 37 times more bacteria per unit surface area on GAC than sand, and that the TOC removal rates were 51% (vs) 26%. Greater biomass levels on GAC (vs) anthracite were also correlated with better organic carbon removals (52). Although correlations between biomass and filter performance were established in the above cases, Urfer and coworkers (53) contend that as long as the biomass level exceeds a minimum concentration, cell numbers do not limit filter performance. The ability to retain this biomass may be material-related, however, because GAC can remove potentially inhibitory compounds (8), sequester organic molecules for growth (12,54) and may protect bacteria from removal by abrasion because of its microporous structure. Another practical advantage of GAC over other media is that the attached microbial population is less prone to shock from changes in water quality, down time, or accidental application of disinfectant (55,56).

Ozonation before biological filtration is often practiced because it frequently improves the biological removal of the smaller molecular weight organic compounds created during oxidation of the natural organic matter (46,57). Preozonation can also result in increased biomass within the filters, most likely because of a higher concentration of usable organic matter (53,58). This effect is not universal, however, because there are cases where ozone does not enhance the metabolism of the organic carbon (59). It has also been observed that populations developing in biological filters fed ozonated water are different from those given nonozonated water. Microbial communities established on nonozonated water used a wider variety of carbon sources at all depths in the filters. In contrast, there was a greater segregation in substrate utilization specificity with depth in filters that were exposed to ozonated water (60).

Because ozonation tends to produce a variety of easily utilized organic compounds, microbial communities in biological filters generally exhibit greater activity at the top of the filters (58,61). The stratification is due to the rapid utilization of the easily degradable organic matter in the top of the filter, with the subsequent dependence on degradation of more recalcitrant compounds as depth and hydraulic residence time increases. This activity may be characterized by both a decrease in the number of carbon substrates used and a change in population with increasing filter depth as determined using phospholipid fatty acid profiles. In the same experimental system, arbitrarily primed PCR sequencing also identified a higher diversity of organisms in the tops of the filters (60). The biomass profile through a filter has been described as first order, and if the filter is sufficiently deep, a portion of it will not be contributing to the biological removal of organic carbon constituents (61).

The potential for a wide range of metabolic functions of the microbial communities in biological filters is further exemplified by the multiplicity of compounds that can be removed from the water. Micropollutants such as phenol can be metabolized when fed simultaneously with ozonated natural organic matter (50). This effect also occurs in simulated wastewater situations, where a biofilm on GAC degraded a mixture of phenols, *N*-heterocyclic compounds and aromatic amines (62). It is also possible to reduce bromate to bromide in biological filters, providing

that the competitive effects of nitrate and oxygen as alternative electron acceptors are controlled (63).

THE PERSISTENCE OF PATHOGENS AND INDICATOR ORGANISMS ON GAC

There has been apprehension expressed by the drinking water industry over the potential proliferation and/or concentration of organisms of public health concern on granular and biologically active carbon filters. As noted earlier, coliforms can become a component of the microflora on filter media. Because biological filtration encourages the growth of bacteria on the filters, it is important to know whether indicator organism and pathogen growth would be enhanced along with the general heterotrophic population.

To address this concern, the potential for coliforms to persist on laboratory columns with virgin GAC, GAC that had been in operation in a filter for a few months, and biologically activated carbon (BAC) from a full-scale plant, was evaluated. The columns were challenged with a suite of five coliforms that had been originally isolated from drinking water distribution systems. The release of the coliforms was tracked by enumerating the organisms in the column effluent. The loss of coliforms in the water for fresh GAC was 0.12 to 0.16 log/day and 0.12 to 0.25 log/day for BAC. The coliforms were detected in the column effluent for approximately one month, although up to 100 mL was filtered to obtain a positive result. Overall, the loss rate from the biologically active carbon was the most rapid of the three media tested. At the end of the experiments, carbon samples were processed and the numbers of coliforms per gram dry mass GAC were determined. Levels were highest on the fresh GAC, intermediate for the precolonized GAC and lowest for the BAC. Conversely, the BAC had the highest concentration of heterotrophic bacteria (38). These results suggest that there is competitive exclusion of the coliform bacteria on the BAC and that the presence of initially attached heterotrophs may decrease the potential for the long-term persistence of undesirable organisms.

In studies performed using BAC filters inoculated with *Pseudomonas aeruginosa*, *P. putida* and *Escherichia coli*, Rollinger and Dott (14) showed that all of these organisms were eliminated from the column between ten and sixteen days. In the same experiments, fresh GAC was colonized with *E. coli*, *K. pneumoniae*, *S. faecalis*, *P. aeruginosa* and *P. putida*. The two pseudomonads and *E. coli* colonized the filter media, whereas the other two organisms were eliminated. These data suggested that perhaps antagonistic compounds were produced by the autochthonous bacteria in the BAC, but less than 1% of the tested strains inhibited the test organisms. Even though the mechanism was not discovered, the observation that colonized BAC reduces the potential for long-term persistence of organisms of public health was confirmed in this study.

The potential growth of coliforms on filter media is of regulatory concern, but has little direct public health significance. However, because coliforms can colonize and persist on GAC filters, there has been the concern that various pathogens may act in a similar manner. The drinking water industry is interested in the survival on GAC and BAC filters of both opportunistic (causing disease only in the immunocompromised individual) and primary pathogens (causing disease in the entire population providing that the infective dose is sufficiently large).

A review of opportunistic pathogens in drinking water has been prepared by Geldreich (64). This review lists a wide variety of organisms that are known to be components of the general heterotrophic population. As such, the presence of these bacteria in drinking water treatment and distribution systems can be expected. Two examples are *Klebsiella* spp. and *Aeromonas* spp. Both of these organisms are capable of expressing virulence factors for human infections that are not waterborne (65–67), and both have been found in granular activated carbon filters (13,68). Others that belong to this group are the *Mycobacterium avium* complex (MAC) and *Legionella* spp. Both are known to grow in biofilms in distribution systems (28,69–73), but there have been no documented reports of their presence in filter media.

In contrast to the opportunistic pathogens, the primary pathogens are not natural components of environmental systems; they are more likely to be found in the gastrointestinal system of a warm-blooded host. To colonize and persist on filter media, these pathogens must be able to successfully compete with the heterotrophic bacterial populations. As with the coliform bacteria, competition with the existing microflora may be a key parameter in preventing proliferation of these bacteria. In a study where the ability of primary pathogens to persist on granular activated carbon was assessed, three enteric pathogens (*Yersinia enterocolitica*, *Salmonella typhimurium*, enterotoxigenic *Escherichia coli*) were added to: (*1*) fresh GAC as pure cultures, (*2*) fresh GAC in the presence of unsterilized river water containing naturally occurring bacteria, and (*3*) GAC previously colonized with river water bacteria (74). Using cultural methods, the greatest level of persistence of the pathogens was on the fresh GAC with sterile river water. Survival decreased when the natural organisms were present, and was the lowest on the previously colonized GAC. Pathogens could be detected in the column effluent by culturing for more than 20 to 24 days when introduced to fresh GAC but were eliminated between 8 and 14 days if the support medium was precolonized GAC. The rate of elimination on the precolonized GAC was 0.11 to 0.7 log per day. Other laboratory studies (14) with several pathogens on GAC reported similar results. The pathogens persisted when introduced to sterile GAC and fed sterile water, but were eliminated from the medium when they were subsequently challenged by autochthonous bacteria in tap water. These data demonstrated that colonized GAC was likely to be beneficial in preventing the proliferation of pathogens.

In the earlier experiments, detection of the pathogens was limited to culturing techniques, which are known to underestimate the numbers of organisms present. Subsequent work has used cultural media combined with direct microscopy and polymerase chain reaction (PCR) methods to follow the fate of bacterial pathogens (*E. coli* O157 : H7 and *S. typhimurium*) in laboratory biologically

active filter media columns. Suspensions of the pathogens were circulated through the columns for 24 hours followed by a continuous flow of dechlorinated tap water with and without relevant concentrations of ozonation by-products. The ozone by-products (aldehydes, etc.) were supplied to determine whether the potential for pathogen survival increased if more degradable low molecular weight compounds were present in the water. Column effluent was sampled routinely, and filter media cores were taken at the end of the experiment. Semi-quantitative PCR showed that there was a 90% reduction in pathogens in the recycled column inoculum before initiation of the tap water feed. These results were mirrored by fluorescent antibody direct counts for *E. coli* O157 : H7. One may assume the reduction is due to attachment to the filter media, because the control system (batch system, no exposure to filter media) showed no decrease in cell numbers over the same period. When throughflow was resumed, no culturable pathogens were detected, and PCR and direct counts using fluorescent antibodies indicated a decline of cells in effluents over the 2.5-week duration of the experiment. After this time, filter media were directly examined for the presence of pathogens. PCR amplification from DNA extracted from the filter media were positive for both *S. typhimurium* and *E. coli* O157 : H7 in one replicate experiment and only for *E. coli* O157 : H7 in the second run, although the signal intensity was quite low. Columns that were supplied additional organic carbon in the form of ozonation by-products did not have enhanced pathogen bacterial persistence or proliferation (68).

RELEASE OF BACTERIA AND COLONIZED PARTICLES FROM GAC FILTERS

As water passes through GAC filters, particularly those that are optimized for biological activity, a significant number of bacteria can be released. The increase in the numbers of organisms from filter influent to effluent can be two orders of magnitude (75). These organisms may be released as single cells, aggregates, or attached to filter media particles (filter fines) that escape through the underdrain of the filters.

In field studies, Camper and coworkers (76) collected released filter fines from GAC filters by passing water from the underdrain during an entire filter run through a gauze filter in a 47 mm Swinnex. The average volume sampled was 18,600 liters. The number of released carbon fines in 201 samples was determined using image analysis. Recognizing that small fines may not have been efficiently retained in the gauze, the average number of particles in a sample was 2,333 ranging in size from 1.0 to $3.5 \times 10^3 \mu m$ in diameter. The bacteria were desorbed from the surface (24) and the number of heterotrophs and coliforms determined by plate count. The results indicated that 17% of the filter runs released carbon fines that contained coliform bacteria, and 28% of these coliforms exhibited the fecal biotype. It should be noted, however, that none of these utilities experienced elevated coliform numbers in their distribution systems. In a similar study, Stewart and coworkers (77) used a modified Swinnex with a polycarbonate filter to trap carbon particles released from a pilot GAC filter. An average of 36 particles per liter were detected with sizes ranging from 2 to greater than 40 micrometers. Using the homogenization technique it was determined that 200 to 7,000 viable bacteria could be recovered from 1,000 particles. Numbers of coliforms were low, with one reported fecal coliform isolated from the released filter fines.

The type of filter media and filter operations may influence the release of colonized filter particles. In a comparison between bacterial counts on released particles from biologically activated sand, anthracite, and biologically activated carbon, the BAC particles contained significantly higher numbers of heterotrophs (49). When the release of particles from full-scale anthracite and sand filters was evaluated (76), particle counts were similar to those from GAC filters. In the same study, where particles released from GAC filters throughout a filter run were enumerated, higher filtration rates, deeper GAC filter beds, and higher applied water turbidity resulted in higher particle release. The age of the carbon was not an important factor. In a similar study, Stringfellow and coworkers (78) demonstrated that the number of particles released from sand or GAC contactors were similar. They also observed that these particles were frequently colonized by heterotrophs, although no coliforms were detected.

Amirtharajah and Westein (79) showed that an elevated number of particles were released both immediately before backwash and shortly after the filter was put back in operation if proper filter-to-waste procedures were not followed. It is probable that colonized filter fines or detached bacteria were also released during these two times, as illustrated by an elevated number of coliforms detected in the filtrate immediately after backwash (33). More recently, Moran and coworkers (80) showed that there can be breakthrough of turbidity due to detachment of particles from deep laboratory filters at the end of a filter run, and these particle sizes were comparable to the range associated with *Cryptosporidium* oocysts.

DISINFECTION OF ATTACHED BACTERIA

The release of bacteria from GAC filters, especially those attached to carbon particles, is important because cells in aggregates or attached to filter media are less susceptible to disinfection than single cells (13,17,24,77). The level of protection appears to be dependent on the size of the fine (78), with bacteria associated with larger particles less susceptible to disinfection than those attached to particles of approximately the same size as the bacterium. The lack of disinfection efficacy against particle-associated bacteria may be the result of biofilm formation on the surfaces. It has been demonstrated that biofilms in general are far less susceptible to disinfectants than single suspended cells (81). In the case where oxidizing disinfectants are used, the phenomenon has been explained as a reaction-diffusion issue. The oxidizing partially disinfectant is consumed in the outer layers of the biofilm, resulting in levels insufficient to kill cells at the base of the biofilm (82,83). However, this phenomenon is inadequate

to explain the apparent lack of disinfection efficacy against very thin biofilms seen on GAC particles. Work by Srinivasan and coworkers (84) showed that thin biofilms were resistant to monochloramine, and suggest that physiological changes in these cells is responsible for the decreased sensitivity.

Regardless of the mechanism, the magnitude of this reduced sensitivity has been demonstrated in many experiments. LeChevallier and coworkers (13) showed that GAC-associated bacteria were not affected by exposure to 2-mg/L free chlorine for one hour. In similar experiments, free chlorine or monochloramine at concentrations of 1.5 mg/L for a 40-minute exposure time did not decrease the plate counts of GAC attached cells (77). Camper and coworkers (24) treated filter fines from operating GAC filters with 2-mg/L free chlorine for 30 minutes, and still recovered culturable cells from the GAC particles. In experiments where free chlorine and monochloramine were compared, monochloramine provided better log reduction of GAC attached cells only if the concentrations of both disinfectants exceeded 2 mg/L (38).

Even though particle-associated bacteria have been shown to be less susceptible to disinfectants, the quality of water produced by GAC filters has not been shown to be compromised. In a study where GAC pilot system effluent samples containing coliforms in the range of <1 to 300 CFU/100 ml were exposed to 2.5-mg/L free chlorine for 30 minutes, no coliforms were detected in 62 of 64 samples. For the two positive samples, the maximum detected number was 7/100 ml. In the same study, heterotrophic plate counts were reduced from an average of 58 CFU/ml to <1–4/ml (85). Similarly, the efficacy of disinfection downstream from biological filters in reducing microbial loads in finished water has been documented in both research and utility settings (35,36,38,47,49,55).

IMPLICATIONS FOR DISTRIBUTION SYSTEMS

It is known that heterotrophic bacteria carried by particles can penetrate the disinfection barrier, attach to the pipe surface, grow, and develop into a biofilm; this becomes critical if pathogens are involved (86,87). With the evidence mentioned earlier that particle associated bacteria are less susceptible to disinfection in mind, utilities have concerns about the possible transport of organisms on filter fines through the disinfection barrier to the distribution system and subsequent regrowth of undesirable bacteria.

To determine if particles can be transported to biofilms, mixed population drinking water biofilm reactors were challenged with slug dose inoculations of carbon particles in the size range of either 1.2 to 8μm or 1.2 to 50μm in diameter. The retention of the carbon fines in the biofilms over time in the presence and absence of disinfection was followed using microscopy techniques. A low percentage (20%) of the small fines initially attached to the biofilm. In contrast, nearly 70% of the larger fines were retained, resulting in a 10-fold increase in carbon mass in the biofilm over that seen with the smaller carbon fines. The larger fines were retained more effectively in the biofilm, but a reduction of nearly 80% of the particles was observed over the three-week experiment. In addition to specific information on the carbon fines, general observations on the response of biofilms to monochloramine (1 mg/L influent concentration) and free chlorine (0.5 mg/L influent concentration) were obtained. Although it took considerably longer, monochloramine ultimately decreased the number of culturable biofilm cells to essentially the same level as free chlorine. The reduction of culturability by approximately three logs was not reflected in loss of viability as assessed by staining techniques; this decrease was only one log. Neither disinfectant caused significant loss nor detachment of biofilm cells from the surface as documented by total cell counts. As determined using activity stains, the biofilm organisms in proximity of the carbon particles did not exhibit reduced susceptibility to the disinfectants, nor was there an accumulation of bacteria around the fines. These results suggest that large particles can be retained in biofilms, but that their effect on the biofilm ecology may be limited (88).

These studies were then extended to investigate the potential for previously colonized carbon particles to act as a mechanism for transporting bacteria to an established biofilm. The larger fines were incubated in the presence of *K. pneumoniae* and then added as a slug dose to biofilm reactor systems. The coliform was monitored in the biofilm using a monoclonal antibody and epifluorescence microscopy. The particles did transport the bacteria to the biofilm surface, resulting in colonization. Over the three-week period, the coliform did not appear to replicate, nor were there cells detected in areas other than the proximity of the carbon particles. If free chlorine was added, there was preferential removal of the coliforms and carbon fines over that of the general heterotrophic population, but 10% of the initially introduced organisms and 20% of the carbon fines remained in the biofilm. Because the detection method was a monoclonal antibody, there is no information available about the culturability or viability of the remaining target bacteria (89).

These findings answer some of the concerns that utilities have had over the release of colonized filter fines from GAC filters into distribution systems. Even if the fines are colonized and attach to the biofilm in numbers comparable to the numbers of culturable heterotrophs present, there is no apparent long-term impact on the biofilm. Disinfection preferentially removed the carbon particles, and there was no dramatic proliferation of an environmental coliform in the biofilm.

CONCLUSION

With growing demands on water resources and the need to respond to increasingly stringent water quality regulations, the use of GAC and BAC will become more widespread in drinking water treatment. GAC will become colonized with organisms, and this is encouraged when BAC treatment is utilized. For biological treatment, BAC appears to be the support medium of choice in most cases because of higher biomass and greater resiliency

against shocks to the system. Colonization of GAC with indigenous organisms appears to reduce the potential for long-term persistence by pathogenic bacteria, although indicator organisms and opportunistic pathogens may become a component of the ecosystem within the filter. Even though disinfectant-resistant particle-associated bacteria are released from properly operated filters and these particles may associate with distribution system biofilms, typical disinfectant regimes have controlled these potentially deleterious effects. On the basis of the results of research and practical experience, it is apparent that the overall benefits of granular activated carbon filtration and biological filtration far outweigh the concerns that may be expressed about deleterious microbiological impacts on potable water.

BIBLIOGRAPHY

1. M. T. Suidan, V. L. Snoeyink, and R. A. Schmitz, *Environ. Sci. Technol.* **11**, 785–789 (1977).
2. M. T. Suidan, V. L. Snoeyink, and R. A. Schmitz, *J. Environ. Eng. Div. ASCE.* **103**, 677–691 (1977).
3. R. C. Bauer and V. L. Snoeyink, *J. Water Pollut. Control Fed.* **45**, 2,290–2,301 (1973).
4. A. S. C. Chen, R. A. Larson, and V. L. Snoeyink, *Environ. Sci. Technol.* **16**, 268–273 (1982).
5. M. C. Lee, V. L. Snoeyink, and J. C. Crittenden, *J. Am. Water Works Assoc.* **73**, 44–49 (1981).
6. D. P. Wilcox, E. Chang, K. L. Dickson, and K. R. Johansson, *Appl. Environ. Microbiol.* **46**, 406–416 (1983).
7. J. Kucera, *Chem. Eng. Prog.* **93**, 54–61 (1997).
8. M. Klotz, P. Werner, and R. Schweisfurth, in *Translation of Reports on Special Problems of Water Technology*, EPA-600/9-76-030, U.S. Environmental Protection Agency, 1976.
9. H. Sontheimer, *J. Am. Water Works Assoc.* **70**, 393–398 (1978).
10. D. van der Kooij, in *Translation of Reports on Special Problems of Water Technology*, EPA-600/9-76-030, U.S. Environmental Protection Agency, 1976.
11. A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, *J. Microbiol. Methods* **3**, 187–198 (1985).
12. D. G. Davies and G. A. McFeters, *Microb. Ecol.* **15**, 165–175 (1988).
13. M. W. LeChevallier, T. S. Hassenauer, A. K. Camper, and G. A. McFeters, *Appl. Environ. Microbiol.* **48**, 918–928 (1984).
14. Y. Rollinger and W. Dott, *Appl. Environ. Microbiol.* **53**, 777–781 (1987).
15. H. T. Chang and B. E. Rittmann, *Environ. Sci. Technol.* **21**, 250–255 (1987).
16. G. E. Speitel, Jr. and F. A. DiGiano, *J. Amer. Water Works Assoc.* **79**, 64–69 (1987).
17. F. Parsons, in report from United States Environmental Protection Agency, Cincinnati, Ohio, 1980.
18. M. W. LeChevallier, W. C. Becker, R. G. Lee, and P. Schorr, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1991.
19. M. Prevost et al., in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1995.
20. J. Z. Wang and R. S. Summers, in *Proc. AWWA Annual Meeting*, American Water Works Association, Denver, Col., 1995.
21. W. J. Weber, Jr., M. Pirbazari, and G. L. Melson, *Environ. Sci. Technol.* **12**, 817–819 (1978).
22. P. R. Cairo, J. McElhaney, and I. H. Suffet, *J. Amer. Water Works Assoc.* **71**, 738–740 (1979).
23. R. J. Shimp and F. K. Pfaender, *Appl. Environ. Microbiol.* **44**, 471–477 (1982).
24. A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, *Appl. Environ. Microbiol.* **52**, 434–438 (1986).
25. M. M. Bourbigot, *Trib. Cebedeau* 448(34), 131–135 (1981).
26. G. A. Burlingame, I. W. Suffet, and W. O. Pipes, *Can. J. Microbiol.* **32**, 226–230 (1986).
27. J. G. de Blanken, *J. Environ. Eng. Div. Soc. Civil Eng.* **108**, 405–410 (1982).
28. C. D. Norton and M. W. LeChevallier, *Appl. Environ. Microbiol.* **66**, 268–276 (2000).
29. B. B. Cummins and H. D. Nash, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1978.
30. W. S. Brewer and W. W. Carmichael, *J. Amer. Water Works Assoc.* **71**, 738–740 (1979).
31. R. S. Tobin, D. K. Smith, and J. A. Lindsay, *Appl. Environ. Microbiol.* **41**, 646–651 (1981).
32. J. M. Symons et al., in *Treatment Techniques for Controlling Trihalomethanes in Drinking Water*, EPA-60012-81-156, U.S. Environmental Protection Agency, 1981.
33. K. E. Bucklin, G. A. McFeters, and A. Amirtharajah, *Water Res.* **25**, 1013–1017 (1991).
34. J. V. Fiore and R. A. Babineau, *Appl. Environ. Microbiol.* **34**, 541–546 (1977).
35. S. D. Faust and O. M. Aly, *Adsorption Process for Water Treatment*, Butterworth Pub Stoneham, MA, (1987).
36. B. E. Rittman and P. M. Huck, *CRC Reviews in Environ. Control* **19**, 119–184 (1989).
37. M. Prevost, R. Desjardins, D. Duchesene, and C. Poirier, *Environ. Technol.* **12**, 569–580 (1991).
38. M. W. LeChevallier et al., *Microbial Impact of Biological Filtration*, AWWA Research Foundation and AWWA, Denver, Col., 1998.
39. P. Servais, P. Laurent, and G. Randon, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1991.
40. H. Sontheimer, *J. Amer. Water Works Assoc.* **71**, 612–617 (1979).
41. H. Sontheimer, *J. Amer. Water Works Assoc.* **71**, 618–622 (1979).
42. E. J. Bouwer and P. B. Crowe, *J. Amer. Water Works Assoc.* **80**, 82–87 (1988).
43. D. van der Kooij, *Biological Processes in Carbon Filters. Activated Carbon in Drinking Water Technology*, KIWA/AWWARF Report, Denver, Col., 1983.
44. R. Ahmad, A. Amirtharajah, A. A. Shawwa, and P. M. Huck, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1994.
45. M. Eberhard, S. Madsen, and H. Sontheimer, *Investigations of the use of Biologically Effective Activated Carbon Filters in the Processing of Drinking Water*, U.S. Environmental Protection Agency, EPA-TR-77-503, Cincinnati, Ohio, 1977.
46. H. Sontheimer and C. Hubele, in P. M. Huck and P. Tofts, eds., *Treatment of Drinking Water for Organic Contaminants*, Pergamon, New York, 1987.

47. M. M. Bourbigot, A. Dodin, and R. Lherritier, in *Proc. AWWA Annual Conf.*, American Water Works Association, Denver, Col., 1982.
48. D. van der Kooij and A. M. Hijnen, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1985.
49. M. W. LeChevallier, W. C. Becker, P. Schorr, and R. G. Lee, *J. Amer. Water Works Assoc.* **84**(4), 136–146 (1992).
50. J. E. DeWaters and F. DiGiano, *J. Amer. Water Works Assoc.* **82**, 69–75 (1990).
51. R. M. Hozalski, S. Goel, and E. J. Bouwer, *J. Amer. Water Works Assoc.* **87**(12), 40–54 (1995).
52. P. Niquette et al., *J. Amer. Water Works Assoc.* **90**(1), 86–97 (1998).
53. D. Urfer, P. M. Huck, S. D. J. Booth, and B. M. Coffey, *J. Amer. Water Works Assoc.* **89**(12), 83–98 (1997).
54. AY. L. Li and F. DiGiano, *J. Water Pollut. Control Fed.* **55**, 392–396 (1983).
55. G. P. Bablon, C. Ventresque, and R. B. Aim, *J. Amer. Water Works Assoc.* **80**(12), 47–53 (1988).
56. S. W. Krasner, M. J. Sclimenti, and B. M. Coffey, *J. Amer. Water Works Assoc.* **85**(5), 62–71 (1993).
57. R. Brunet, M. M. Bourbigot, and M. Doré, *Ozone Sci. Technol.* **4**, 15–21 (1982).
58. J. Z. Wang, R. S. Summers, and R. J. Miltner, *J. Amer. Water Works Assoc.* **87**(12), 55–63 (1995).
59. W. H. Glaze et al., in *Evaluation of Biological Activated Carbon for Removal of Trhihalomethane Precursors*, Report to the U.S. Environmental Protection Agency, US. EPA, Cincinnati, Ohio, 1982.
60. D. M. Moll, R. S. Summers, and A. Breen, *Appl. Environ. Microbiol.* **64**, 2755–2759 (1998).
61. K. H. Carlson and G. L. Amy, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1995.
62. B. Koch, M. Ostermann, H. Hoke, and D.C. Hempel, *Water Res.* **25**, 1–8 (1991).
63. M. J. Kirisits and V. L. Snoeyink, *J. Amer. Water Works Assoc.* **74**(8), 74–84 (1999).
64. E. E. Geldreich, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1991.
65. J. M. Janda, *Clin. Microbiol. Rev.* **4**, 397–410 (1991).
66. S. J. Gray, D. J. Stickler, and T. N. Bryan, *Epidemiol. Infect.* **105**, 277–294 (1990).
67. S. T. Bagley and R. J. Seidler, *Appl. Environ. Microbiol.* **33**, 1141–1148 (1977).
68. M. Burr, A. K. Camper, P. Hacker, and R. DeLeon, *Colonization of Biologically Active Filter Media with Pathogens*, AWWA Research Foundation and AWWA, Denver, Col. (In press).
69. C. H. Collins, J. M. Grange, and M. D. Yates, *J. Appl. Bacteriol.* **57**, 193–211 (1984).
70. E. G. Means, K. N. Scott, M. L. Lee, and R. L. Wolfe, in *Proc. AWWA Annual Conf.*, American Water Works Association, Denver, Col., 1986.
71. L. A. Briganti and S. C. Wacker, *Fatty Acid Profiling and the Identification of Environmental Bacteria for Drinking Water Utilities*, American Water Works Association, Research Foundation, Denver, Col., 1995.
72. J. Rogers and C. W. Keevil, *Appl. Environ. Microbiol.* **58**, 2326–2330 (1992).
73. J. S. Colbourne et al., in *Proceedings International Conference on Water and Wastewater Microbiology*, Newport Beach, Calif., 1988.
74. A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, *Appl. Environ. Microbiol.* **50**, 1378–1382 (1985).
75. R. Ahmad and A. Amirtharajah, in *Proc AWWA Annual Meeting*, American Water Works Association, Denver, Col., 1995.
76. A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, *J. Amer. Water Works Assoc.* **79**, 70–74 (1987).
77. M. H. Stewart, R. L. Wolfe, and E. G. Means, *Appl. Environ. Microbiol.* **56**, 3822–3829 (1990).
78. W. T. Stringfellow, K. Mallon, and F. A. DiGiano, *J. Amer. Water Works Assoc.* **85**, 70–80 (1993).
79. A. Amirtharajah and D. P. Westein, *J. Amer. Water Works Assoc.* **72**, 518–524 (1980).
80. C. M. Moran, D. C. Moran, R. S. Cushing, and D. F. Lawler, *J. Amer. Water Works Assoc.* **85**, 82–93 (1993).
81. J. W. Costerton, *Dev. Ind. Microbiol.* **25**, 363–37 (1984).
82. P. S. Stewart, *Antimicrob. Agents Chemother.* **38**, 1052–1058 (1994).
83. H. Tashiro, T. Numakura, S. Nishikawa, and Y. Miyaji, *Water Sci. Technol.* **23**, 1395–1403 (1991).
84. R. Srininvasan et al., *Biotechnol. Bioeng.* **46**, 553–560 (1995).
85. K. W. Himmelstein, D. J. Hartman, and J. DeMarco, in *Proc. Water Qual. Technol. Conf.*, American Water Works Association, Denver, Col., 1990.
86. M. W. LeChevallier, *J. Amer. Water Works Assoc.* **82**, 74–86 (1990).
87. R. McEnroe, J. E. Tobiason, and M. S. Switzenbaum, in *Proc. Water Qual. Technol. Conf.*, Amer. Water Works Associates, Denver, Col., 1992.
88. P. Morin, and A. K. Camper, *Water Res.* **31**, 300–410 (1997).
89. P. Morin et al., *Appl. Environ. Microbiol.* **62**, 4428–4432 (1996).

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See REMOVAL OF PATHOGENIC MICROBES BY GRANULAR HIGH-RATE FILTRATION

GRANULAR SLUDGE.

See ANAEROBIC GRANULES AND GRANULATION PROCESSES

GREEN FLUORESCENT PROTEIN (GFP)

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A powerful tool of molecular microbiology was created with the description of GFP. First described in its natural