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EPA ENVIRONMENTAL RESEARCH BRIEF

Bacteria Attached To Granular Activated Carbon In Drinking Water

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Laboratory and field studies were undertaken to answer basic questions about the influence of granular activated carbon (GAC) on the bacteriological quality of drinking water. A sampling apparatus consisting of a 47-mm Swinnex* and a 16-layer gauze filter was developed to trap filter fines from large volumes of water. A desorption technique (Zwittergent 3-12, 10^{-6}M; EGTA, 10^{-3}M, peptone, 0.01%; Tris buffer, pH 7.0, 0.1M; homogenized at 4°C for 3 min at 16,000 rpm) combined with optimal culturing procedures (heterotrophs, R2A medium at 28°C for 7 days; coliforms, mT7 medium MF procedure and an MPN with lauryl sulfate added after 4 hr of incubation) allowed for the enumeration of particle-associated bacteria

GAC-attached bacteria were resistant to 2.0 mg/L chlorine after 1 hr of exposure. Enteric pathogens were capable of colonizing laboratory-scale GAC filters. Their colonization potential and longevity depended on the presence of autochthonous river water organisms. GAC filter particles were found in effluents from properly operated treatment facilities. More than 40% of the samples obtained contained particles significantly colonized with heterotrophic plate count bacteria, 17% were populated with coliforms. The appearance of colonized fines was not related to a specific time in filter operation. Increases in the breakthrough of bacteria-laden particles were seen in the spring and fall. Several operational variables (increased bed depth, turbidity of applied water, and filtration rate) did correlate positively with the presence of fines in filter effluents. Bed age was not associated with breakthrough

* Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This Research Brief was developed by the principal investigators and EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in the reports and publications listed at the end.

Introduction

The deterioration of surface water quality and increasing taste and odor problems in drinking water have resulted in the widespread use of activated carbon filtration medium. The active surface area of this substance makes it ideal for the removal of organic molecules, including trihalomethanes (THM's). However, certain characteristics of this compound also make it an ideal substance for the concentration of bacterial nutrients. Bacteria have been shown to adsorb to and extensively colonize the surface of activated carbon particles in granular activated carbon (GAC) filter beds. The seeding of distribution systems with bacteria can occur if colonized particles pass treatment barriers or if the organisms are sloughed or sheared from filters. These cells are not deleterious to water quality if disinfection is adequate, as previous studies have suggested. However, the chlorine resistance of GAC-adsorbed cells has not been adequately addressed.

Prior investigations into the bacteriological impact of GAC filtration have relied on standard grab sample and enumeration procedures. Thus it is possible that a heavily colonized particle would yield only one colony. Some researchers have reported large nmbers of bacteria in GAC-filtered drinking water and others have not. The lack of appropriate methods to enumerate adsorbed cells has contributed to this problem. The development of procedures to release cells from particles, deaggregate

them, and effectively enumerate them is necessary to adequately study the influence of GAC on the bacteriological quality of treated water

If bacteria from GAC filter beds can reach drinking water, the public health significance of these organisms must be considered. Earlier research has identified potential or opportunistic pathogens in GAC-treated water. Research into the ability of pathogens to colonize GAC filters and survive disinfection was undertaken to determine whether pathogenic bacteria could enter drinking water from this source.

If GAC filter material can penetrate treatment barriers, it is important to define how this phenomenon is influenced by variables in the operation of a drinking water filter. Though other investigators have implicated certain operational procedures in the appearance of organisms in finished water, no attempt has been made to identify variables involved in the occurrence of populated filter fines Information from these studies could help plant operators select procedures to minimize breakthrough of colonized filter material.

This summary addresses the following questions related to research conducted in these areas: (1) How can GAC-borne bacteria be accurately enumerated, (2) how susceptible are adsorbed bacteria to disinfection by chlorine, (3) if bacteria of public health significance can colonize GAC filter beds, do colonized particles of filtration media actually appear in finished water, (4) what operational variables contribute to the occurrence of colonized particles in filtered water, and (5) what physiological advantage, if any, do GAC-attached cells have over planktonic cells.

Results and Discussion

Desorption and Enumeration Techniques

Fundamental to this research was the establishment of a procedure to remove bacteria adsorbed to the surface of GAC and to prevent reattachment without compromising bacterial viability. The dispersal of individual cells would then allow for a more accurate determination of the actual bacterial load on waterborne GAC particles

Initial experiments were performed to determine which physical means of interrupting cell-surface interactions were most efficient. Sonication and blending decreased cell viability as a result of heat generation. Optimal counts were attained when the sample was homogenized for 3 min at 16,000 rpm in a container immersed in an ice bath (ca. 4°C). In addition, specific chemicals and enzymes selected for their ability to disrupt extracellular polymeric materials or to act as surface-charge interactors were tested in conjunction with homogenization.

Of the 35 chemicals, combinations, or concentrations evaluated, the greatest numbers of bacteria were detected with a solution of Zwittergent 3-12 (10^{-6} M), ethyleneglycol-bis-(beta amino-ethyl ether)-N,N^1-tetra acetic acid (EGTA) (10^{-3} M), peptone (0.1 %), and Tris buffer (0.1 mM, pH 7.0). The efficacy of the technique was tested using a known number of cells adsorbed to activated carbon. Approximately 90% of the bacteria were recovered, as determined by plate counts. This result was supported by observation of acridine-orange-treated GAC particles with epifluorescence microscopy.

The medium and growth conditions under which maximum cell counts could be obtained were then determined. Four media (plate count agar, 0.1 plate count agar, mSPC, and R2A) were incubated at 28°C for varying lengths of time (2 to 7 days) for the enumeration of heterotrophic plate count (HPC) bacteria. R2A medium incubated for 7 days at 28°C consistently provided the highest counts. mT7 agar was used as the medium of choice for enumerating desorbed coliforms by the membrane filter (MF) procedure. A modified MPN (3 tubes, 3 dilutions, and lauryl sulfate addition after 4 hr of incubation) was also used as a coliform enumeration technique, as it has been shown that sample turbidity can interfere with MF detection of these organisms.

Susceptibility of GAC-Attached Bacteria to Chlorine

Particle-associated bacteria are reported to be more resistant to disinfection. Thus because colonized GAC filter bed particles could be released from the filter, the chlorine resistance and public health significance of these attached bacteria merit investigation.

Experiments were conducted with GAC removed from an operating drinking water filter and maintained in a column in the laboratory. Planktonic cells cultured from this column were also used. GAC-attached and planktonic cells of an *Escherichia coli* river isolate and the pathogens *Salmonella typhimurium, Yersinia enterocolitica*, and *Shigella sonnei* were also tested. For these experiments, the attachment was accomplished by exposing virgin GAC to suspensions of each bacterial species for 20 min followed by gentle rinsing. In this time, the bacteria had little opportunity to produce extracellular material. Cells were also grown in the presence of GAC to evaluate the chlorine resistance of the GAC-attached biofilms.

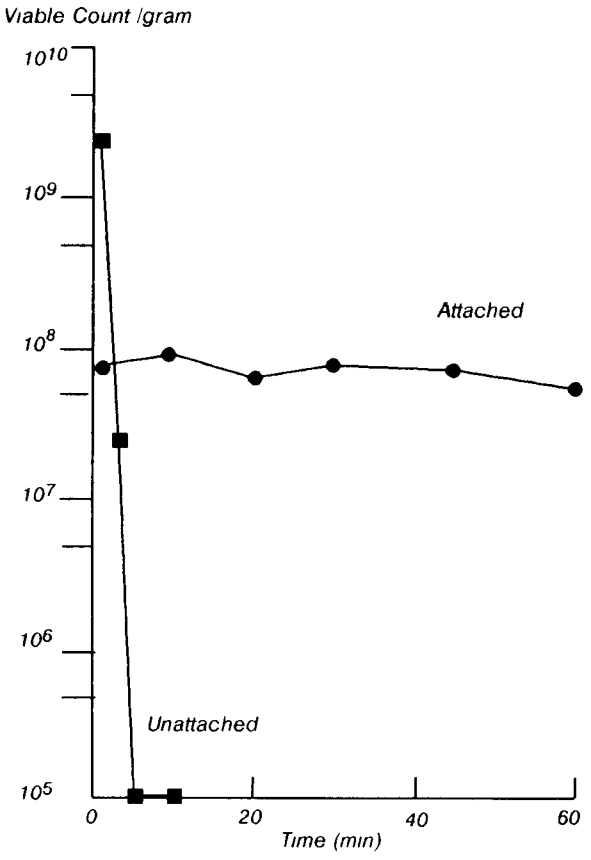
Scanning electron micrographs of these particles and of those from the drinking water filter revealed colonies of bacteria and the presence of extracellular polymer. The exposure of planktonic HPC cells to chlorine (2.0 mg/L) resulted in a rapid decrease in viability (within 5 min), whereas GAC-associated cells experienced little decline in numbers after exposure for 1 hr (Figure 1). No decrease in viability was observed within 1 hr for the GAC-grown coliforms. Some injury did occur with GAC-attached cells, suggesting that the extracellular polymer produced by the grown cells or the integrity of the colony afforded some amount of protection from chlorine.

These data suggest a means by which bacteria, including pathogens, can breach disinfection barriers and enter distribution systems.

Growth and Persistence of Enteric Pathogens on GAC Filters

The potential for colonization of GAC by enteric pathogens (*Yersinia enterocolitica, Salmonella typhimurium*, and a human enterotoxigenic *Escherichia coli*) was investigated. Laboratory GAC columns were inoculated with each organism in the presence or absence of autochthonous river water organisms. Core samples were removed from the columns at regular intervals and homogenized.

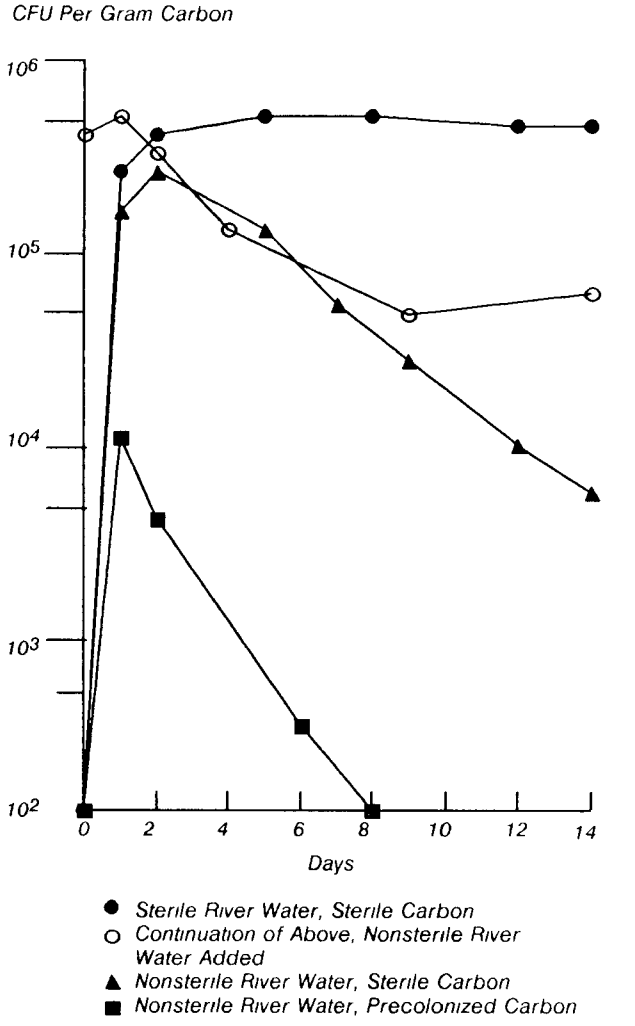
Figure 1. **Survival of naturally occurring heterotrophic plate count bacteria exposed to chlorine at 2.0 mg/L for 1 hour (free chlorine residual after 1 hour was 1.7 mg/L).**



Bacteria were enumerated by the spread plate technique on plate count agar (HPC) and a selective medium that had been shown to provide maximum counts of each pathogen. When each pathogen was suspended in sterile river water and introduced to a sterile GAC column, the GAC was rapidly colonized Maximum colonization (ca. 10^5 to 10^7 cfu g^{-1} GAC) had occurred by the first sampling time (2 days) and remained for the duration of the experiment (14 to 20 days). When these columns were then exposed to nonsterile river water, the population of the pathogen declined gradually (0.08 to 0.14 log day^{-1}), and pathogens existed (10^4 to 10^6 cfu g^{-1} GAC) at the termination of the experiment. The addition of pathogens to nonsterile river water circulated through an initially sterile column resulted in colonization by pathogens at rates similar to those obtained with sterile water. However, the pathogen numbers declined at a more rapid rate (0.10 to 0.22 log day^{-1}), than when colonization was established before the addition of HPC bacteria. If the pathogens were introduced into a column supporting a mature biofilm of HPC bacteria, there was least attachment (10^4 cfu g^{-1} GAC) In this case, pathogen cells attached to the GAC declined at a more rapid rate (0 11 to 0.70 log day^{-1}).

Data from the experiments with *Salmonella typhimurium* are shown in Figure 2. The results demonstrate the

Figure 2. **Attachment and persistence of *S. typhimurium* on GAC Columns.**



importance of indigenous surface water organisms in the control of human enteropathogenic organisms on GAC. An established biofilm of heterotrophic bacteria appears to be beneficial in controlling the attachment and longevity of pathogens on GAC filters.

Colonized Filter Fines in Drinking Water

The occurrence of populated GAC filter fines in drinking water was substantiated at nine operating drinking water treatment facilities A sampling device was developed to allow the testing of large volumes of drinking water The apparatus consisted of a 47-mm Swinnex filter holder with the ends bored to a 6-mm inside diameter. A sterile, 16-layer gauze filter was enclosed in the unit The samplers were installed in each treatment facility at a point after GAC filtration and before final chlorination. GAC filter effluent was passed through the gauze for the entire filter cycle 1 hr before backwash or 4 hr after backwash. The gauze was then removed and shipped to the lab Particles were chlorinated (2.0 ppm, 30 min) to inactivate planktonic cells, since attached cells were more resistant. Following dechlorination, the sample was split. Half was

homogenized, and the other half was handshaken. A greater-than-twofold-increase in colony-forming units (cfu)/mL in homogenized over handshaken samples was used as an index of significant particle colonization More than 200 gauze filters were received. The trapped particles were examined for attached HPC and coliform bacteria

Forty-one percent of the samples contained GAC particles colonized by HPC bacteria Coliforms were found in association with fines from GAC-filtered water in 17% of the samples (Table 1) Of these, nearly 28% exhibited the fecal biotype Scanning electron micrographs of particles clearly demonstrated bacterial cells and associated extracellular material in surface pits and cracks of the GAC particles Analyses also showed that colonized filter material was released throughout the filter cycle and was not related to turbidity spikes just before and after backwashing. Evaluation of the data on the basis of time of year revealed a distinct seasonal trend in the occurrence of attached coliforms (spring and autumn) This trend was not seen with HPC bacteria Image analysis of the particles released from GAC drinking water filters provided information as to their size and shape. Most particles were nearly spherical. The sizes varied from 1.0 μm to $3\,5 \times 10^3$ μm

Note that all these results were based on samples received from drinking water treatement facilities that were run well and in compliance with established operation regulations. The data show that bacteria attached to carbon fines may be an important mechanism by which microorganisms can pass treatment barriers and enter finished water. Indicator bacteria and potential or opportunistic pathogens were observed on these particles that would not have been enumerated by conventional analysis of drinking water samples

Influence of Operating Variables of GAC-Filters on the Occurrence of Populated Fines in Drinking Water

The effects of treatment differences on the release of filter material were studied at two drinking water treatment facilities. Sampling procedures described previously were used Statistical analyses of data obtained during 1 year of bimonthly sampling revealed that GAC filter bed age (virgin and 1, 2, and 3 years old) does not affect the release of colonized particles. At Plant 1 (Table 2), significantly more HPC-populated particles were observed as GAC bed depth increased from 60 cm to 1.5 m. Coliform bacteria also increased with depth from 6% of the 60-cm samples to 25% of the 1.5-m samples. Colonized particle breakthrough correlated positively with increased mean turbidity of applied water (mean 4.3 ntu versus 2 9 ntu). Filtration rate also proved to be important. As the flow rate doubled from 4.9 to 9.8 m/hr (2 to 4 gpm/ft^2), more filter fines were released, and these particles were populated to a greater extent.

Also investigated was the relative contribution of various filtration media to the appearance of populated particles in finished water Studies conducted in the laboratory showed that columns of sand, anthracite, and three brands of GAC were all colonized to the same level by HPC bacteria. However, GAC columns (regardless of manufacturer) supported nearly a 1-log higher coliform

Table 1. Analysis of Particles Collected from GAC-Treated Effluents.

Item	Heterotrophic Plate Count	Coliform	
		MF	MPN
Total Number of Samples	198	201	191
Number Showing > 2x Increase	82 (41.4)[a]	14 (7.0)	33 (17.2)
Meanfold Increase[b]	8.6	124.3	24.5
Maximum Increase	50.0	1194.0	122.2

[a] Numbers in parenthesis indicate percentage of total samples.
[b] Homogenized versus handshaken analyses

Table 2. Effect of GAC Filter Bed Depth, Applied Water Quality, and Filtration Rate on the Release of Populated GAC Particles into Drinking Water at Plant 1.

Item	Depth		Turbidity (ntu)		Flow Rate (Lpm/m^2)	
	60 cm	1.5 m	2.92	4.32	0.72	1.44
Number of Samples	16	16	16	16	17	16
Mean Filter Rating	3.6	2.3	2.1	2.1	2.0	3.6
Median HPC Ratio[a]	1.39	2.21	1.65	3.04	1.53	3.86
p Value	0.002	0.002	0.004	0.004	0.001	0.001

[a] Ratio of colony-forming units from homogenized values divided by handshaken values from split samples.

(*Klebsiella oxytoca*) load than sand or anthracite Sampling devices were installed at treatment facilities with anthracite and sand filters. When compared with results from GAC-filtered water, the GAC-treated effluents contained more particles colonized with chlorine-resistant organisms.

Physiology of GAC-Grown Klebsiella oxytoca Compared with Planktonic Growth

Laboratory studies showed that the growth rate of *Klebsiella oxytoca* adsorbed on GAC was enhanced up to 10 times that of planktonic cells when the organisms were provided with a negatively charged substrate (glutamate) that could adsorb to the particle surface. No differences were observed when the uncharged substrate glucose was used

[^{3}H]-thymidine was used to assess DNA biosynthesis. GAC-attached cells grown on glutamate (20 0 mg/L) took up to five times more [^{3}H]-thymidine than did unattached cells grown in liquid medium. When [^{3}H]-uridine was used to measure RNA turnover, the GAC-attached cells took up 11 times more [^{3}H]-uridine per cell than their planktonic counterparts

Cell size measurements were performed by differential filtration Planktonic cells grown on glutamate (20.0 mg/L) decreased in size and 62% could pass through a 1.0-µm filter after 9 days. Only 39% of the GAC-attached cells passed through a 1.0-µm filter. The studies indicated that GAC provided an enhanced environment for the growth of *Klebsiella oxytoca* when a charged substrate (glutamate) was present that was adsorbed by the GAC.

Conclusions

The following conclusions can be drawn from this research:

1. GAC-attached bacteria were effectively removed by homogenization at 16,000 rpm at 4°C in a solution of Zwittergent 3-12 (10^{-6}M), EGTA (10^{-3}M), peptone (0.01%) and Tris buffer (10^{-1}M) at pH 7.0 for 3 min. HPC bacteria were best enumerated on R2A medium incubated for 7 days at 28°C. Coliforms were effectively quantified with mT7 agar and a modified MPN procedure.
2. HPC, coliform, and enteropathogenic bacteria grown on GAC or attached for less than a generation time were not killed by exposure to chlorine (2 mg/L) for 1 hr.
3. Enteropathogenic bacteria were capable of colonizing laboratory-scale GAC filters. Persistence of the pathogens depended on the presence of autochthonous surface water organisms.
4. Populated GAC filter fines were found in drinking water from properly operated treatment facilities. HPC and coliform bacteria were detected on particles that were released throughout the filter cycle.
5. Increasing the applied water turbidity, flow rate, and filter depth all caused an appearance of (1) a higher number of released particles, (2) increased bacterial colonization of the particles, or (3) elevated adsorbed coliforms. GAC supported more coliforms than sand or anthracite in laboratory experiments.
6. GAC-attached *Klebsiella oxytoca* had a greater growth rate than planktonic cells in the presence of a charged substrate (glutamate). Other physiological indices showed greater activity in adsorbed cells

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The following publications collectively contain the complete findings of this research project.

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